RESPONSES OF ROTATION-RESISTANT AND ROTATION-SUSCEPTIBLE POPULATIONS OF THE WESTERN CORN ROOTWORM TO TRANSGENIC CORN EXPRESSING CRY TOXINS AND VALIDATION OF A DAMAGE FUNCTION FOR CORN ROOTWORM LARVAE

BY

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DISSERTATION

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ABSTRACT

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a significant insect pest of corn, *Zea mays* L. Yield losses and control costs associated with the corn rootworm complex—which includes the northern (*Diabrotica barberi* Smith & Lawrence) and southern (*Diabrotica undecimpunctata howardi* Barber) corn rootworms as well—exceed an estimated $1 billion annually in the United States. In Chapter 1 of this dissertation, a comprehensive review of scholarly literature pertaining to the western corn rootworm is presented.

The primary goal of the multi-year, multi-state field experiment described in Chapter 2 was to determine if rotation-resistant and rotation-susceptible western corn rootworm larvae differ in their ability to injure transgenic Bt corn roots and cause yield loss. A second goal was to determine if these two populations exhibit differences in emergence or fitness when exposed to Bt corn. A variety of response variables were analyzed, including root injury and yields, as well as cumulative beetle emergence, sex ratios, head capsule widths, and weights. Densities of western corn rootworm beetles were quite low at many of the sites during this experiment. As a result, consistent trends related to root injury and fitness measurements for beetles were difficult to detect. The lack of statistical separation among the various locations and treatments evaluated indicates that many of the variables analyzed are of limited usefulness when densities of western corn rootworm beetles are minimal.

The goal of the greenhouse experiment described in Chapter 3 was to determine if rotation-resistant and rotation-susceptible western corn rootworm larvae differ with respect to survival or development on corn expressing one or more Bt toxins—a single-plant bioassay was used. Corn plants were infested with 225 near-hatch eggs at the V5 growth stage (five leaf collar). Larvae were allowed to develop undisturbed on the root systems for 17 d, after which they were recovered using Berlese funnels. Larvae were counted to estimate mortality; head capsule widths were recorded to assess development. No difference between rotation-resistant and rotation-susceptible larvae with respect to mortality caused by exposure to Bt toxins was observed. Head capsule widths suggest that larvae from these two populations develop similarly when reared on Bt corn. Potential explanations for the observed results are discussed.

An analysis of readily available field trial data used to validate an existing damage function for corn rootworm larvae is reported in Chapter 4. A nested error component model with unbalanced panel data spanning 19

location-years was used to describe the relationship between yield loss and root injury. The model suggests that for each node of roots injured by larvae, a yield loss of approximately 15% is expected. Statistically significant sources of variation included location and experimental error. Variation in weather across sites was likely the principal factor contributing to the significant effect of location. The large experimental error highlights the limitations of using a multi-year, geographically diverse damage function for predicting yield loss on a small scale. Major factors contributing to the variance components estimated by this model are discussed, and techniques for improving future analyses of the damage function for corn rootworm larvae are suggested.

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CHAPTER 1: REVIEW OF SCHOLARLY LITERATURE

PERTAINING TO THE WESTERN CORN ROOTWORM

Life History

Introduction

Corn, *Zea mays* (L.), is an economically important crop grown throughout the world. Corn was first domesticated by the indigenous peoples of Central America (Mangelsdorf 1983) and is currently cultivated for a variety of uses (e.g., animal feed, ethanol production). In 2012, an estimated 170 million ha of corn was planted—the United States accounted for approximately 20% of this area (USDA FAS 2012). The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is a chrysomelid beetle that has been a pest of corn in Central America for approximately 5000 years (Melhus et al. 1954). The adoption of large corn monocultures increased the importance of this pest (Smith and Lawrence 1967). In the United States, the western corn rootworm was historically restricted to west-central portions of the Great Plains (Chiang 1973). However, during the mid- to late twentieth century, this pest spread rapidly toward the east coast. Gray et al. (2009) summarized the range expansion of the western corn rootworm, which was reported in Kansas and Nebraska in the 1940s; South Dakota in the 1950s; Illinois, Iowa, Kansas, Minnesota, Missouri, North Dakota, and Wisconsin in the 1960s; Indiana, Michigan, Montana, Ohio, and Oklahoma in the 1970s; Delaware, Kentucky, Maryland, New Hampshire, New Jersey, Pennsylvania, Virginia, and West Virginia in the 1980s; Connecticut, Georgia, Maine, Massachusetts, New Hampshire, North Carolina, Rhode Island, South Carolina, Tennessee, and Vermont in the 1990s; and Alabama in the 2000s. The corn rootworm complex, which includes the western corn rootworm, the northern corn rootworm (*Diabrotica barberi* Smith & Lawrence), and the southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber), is responsible for an estimated $1 billion in yield losses and control costs annually in the United States (Metcalf 1986). However, Dun et al. (2010) suggested that a more current estimate may be significantly larger.

Although native to the western hemisphere, the range of the western corn rootworm has expanded to include Europe. There have been no less than five independent introductions of this pest from the United States into Europe, including sites in southern England, northern and western France, northwestern Italy, and Serbia (Ciosi et

al. 2008). The western corn rootworm was first detected in Europe in 1992 near Surcin International Airport in Belgrade, Serbia (formerly Yugoslavia) (Berger 2001). Beetles were subsequently detected in Croatia and Hungary in 1995; Romania in 1996; Bosnia and Herzegovina in 1997; Bulgaria and Italy in 1998; Slovakia and Switzerland in 2000; Ukraine in 2001; Austria, the Czech Republic, and France in 2002; and Belgium, the Netherlands, and England in 2003 (Kiss et al. 2005a). Beetles also have been detected in Poland (Boriani et al. 2006). Regional movement of beetles throughout Europe has been attributed to transportation and packaging material (Kiss et al. 2005a). Of the total area (18.2 million ha) infested by this pest, economically significant damage has been limited to approximately 2.65 million ha (Berger 2001). The economic benefit for controlling the spread of the western corn rootworm in Europe may be as great as $674 million annually (Wesseler and Fall 2010). A comprehensive eradication program instituted in Italy successfully reduced densities of beetles (Berger 2001). For the most part, natural enemies endemic to Europe do not attack this pest (Toepfer and Kuhlmann 2004). Crop rotation is largely effective for preventing yield loss and reducing populations of beetles (Kiss et al. 2005b; Boriani et al. 2006).

Eggs

The western corn rootworm overwinters as an egg in the soil; eggs are laid during mid- to late summer (Ball 1957). Chilling is not essential for egg development—some larvae can hatch before the growing season ends (Chiang 1974). However, larvae hatching late in the growing season suffer mortality due to lack of food or the onset of cold temperatures. Diapause enhances overwintering survival and ensures synchronization with corn production (Coats et al. 1986). Diapause is shorter at northern latitudes because the period of time between oviposition and the onset of cold temperatures is shorter than at southern latitudes (Krysan 1982; Coats et al. 1986). While extended diapause (diapause lasting more than one winter) has been well-documented in populations of the northern corn rootworm, this trait occurs at a very low rate (0.14 to 0.21% of eggs) for the western corn rootworm (Levine et al. 1992). As winter approaches, cold temperatures induce chill-quiescence, a state that prevents eggs from completing development.

Although western corn rootworm eggs are well-adapted for surviving cold temperatures, prolonged exposure to temperatures below 0 °C increases mortality (Chiang 1973; Gustin 1981). Egg stage at the onset of cold temperatures affects overwintering success as well; mortality increases when developmentally advanced eggs are

exposed to cold temperatures (Palmer et al. 1977). The insulative properties of soil are important for the overwintering success of eggs. Lack of rain- or snowfall during winter and early spring decreases soil temperature and increases mortality (Calkins and Kirk 1969). Surface residues help protect eggs from fluctuations in atmospheric temperatures (Godfrey et al. 1995). Intermittent warm periods experienced by overwintering eggs are unlikely to terminate diapause prematurely (Gustin 1981).

Larvae

As spring approaches, western corn rootworm eggs experience dry-quiescence and must take-up water to complete embryonic development (Krysan 1978). During post-diapause development, mortality for otherwise viable eggs ranges from 2 to 42% (Weiss et al. 1985; Branson 1987; Fisher 1989). Models for predicting larval hatch rely on soil temperatures and cumulative degree days as inputs (Levine et al. 1992; Schaafsma et al. 1993). Levine et al. (1992) predicted 50% hatch at 426 degree days. The feeding period for larvae typically begins in early June and lasts until late July (Bryson et al. 1953). The onset and duration of this period is influenced strongly by seasonal fluctuations in temperature and soil conditions. Following initial hatch, eclosion proceeds at a rate of 1.8 to 2.9% per day (Musick and Fairchild 1971). Optimum temperatures for larval development range from 18 to 30 °C (Fisher 1986), with a minimum threshold for development estimated at 9 °C (Jackson and Elliott 1988). Males typically develop faster than females. Prolonged periods of soil saturation during hatch prevent larval establishment on host plants (Sutter et al. 1989). However, extremely dry soil conditions prevent larval establishment as well (Oloumi-Sadeghi and Levine 1989; MacDonald and Ellis 1990).

During establishment, most western corn rootworm larvae are located in the upper 10 cm of the root zone (Bergman et al. 1981). Larvae have only a limited capacity for burrowing or moving soil particles (Gustin and Schumacher 1989). As a result, soil characteristics are important for successful larval establishment. Soils with pore sizes of 0.15 mm or smaller limit larval movement (Gustin and Schumacher 1989). Results presented by Turpin and Peters (1971) suggest that larval desiccation and subsequent mortality is greater in sandy soils than in clay soils—soils with a high concentration of sand have poor moisture retention and are generally more abrasive to the larval cuticle.

Use of a carbon dioxide gradient in the soil profile, as a result of root respiration, is the primary manner by which western corn rootworm larvae locate corn roots (Strnad et al. 1986). Larvae exhibit the greatest response to carbon dioxide at concentrations ranging from 2.5 to 4.2 mmol/mol—its concentration near corn roots is approximately 4.3 mmol/mol (Bernklau and Bjostad 1998a). Larvae display toxic symptoms at concentrations beyond 300 mmol/mmol. A number of other compounds were proposed as larval attractants during the early 1990s. These included 6-methoxy-2-benzoxazolinone (MBOA) (Bjostad and Hibbard 1992) and various long-chain fatty acids (Hibbard et al. 1994). However, the attractiveness of these compounds was later retracted because the solvent in which they were tested (dichloromethane) was itself attractive to larvae (Bernklau and Bjostad 1998b). Carbon dioxide remains the only volatile attractant identified for larval host location. Corn roots produce at least one compound that acts as a feeding deterrent (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, DIMBOA) (Davis et al. 2000). DIMBOA is produced when larvae injure root tissue and has antibiotic activity.

Western corn rootworm larvae display two distinct types of foraging behavior (Bernklau et al. 2009). When exposed to host tissue, larvae exhibit a highly convoluted, localized foraging behavior. Conversely, when exposed to non-host tissue, larvae exhibit a ranging behavior (relatively straight movement through the soil). Larvae can range at least 100 cm through soil to locate host tissue (Suttle et al. 1967; Short and Luedtke 1970). Larvae shift from ranging to localized foraging within five minutes of contacting host tissue (Strnad and Dunn 1990). Successful larval establishment on a suitable host is important—prolonged starvation increases mortality and decreases the ability of larvae to penetrate root tissue (Branson 1989). Starvation for 24 to 72 h results in larval mortality rates as great as 48 to 100% (Weiss et al. 1985; Oloumi-Sadeghi and Levine 1989). Movement of larvae throughout the soil profile is strongly influenced by developmental age; older larvae more likely to leave their initial feeding site for a more acceptable feeding site than younger larvae (Schumann and Vidal 2012).

After successful establishment, western corn rootworm larvae are subjected to both intra- and interspecific competition (Woodson 1994). In Illinois, western and northern corn rootworm larvae commonly compete for shared resources. Larvae of these two species can be differentiated by measuring anatomical differences in their head capsules (Becker and Meinke 2008) or via genetic techniques (Roehrdanz 2003). Piedrahita et al. (1985) noted that the presence of western corn rootworm larvae reduced survivorship and affected the spatial distribution of northern corn rootworm larvae. With respect to adult emergence, Gray and Tollefson (1988b)

estimated that the western corn rootworm can outcompete the northern corn rootworm by a ratio of 4:1 when similar egg densities of these two species occur simultaneously in the field. Crowding is important for determining the role of intraspecific competition. Density-dependent mortality begins at approximately 2500 viable eggs/m of row (Hibbard et al. 2010b). Increased larval densities negatively affect the number, size, longevity, and fecundity of beetles (Branson and Sutter 1985). As densities of viable eggs increase, larval survival to adulthood decreases (Onstad et al. 2006). Declining food resources that coincide with crowding increase post-establishment larval movement (Hibbard et al. 2004).

In addition to competition, host characteristics and phenology influence western corn rootworm larvae in a number of ways. Corn hybrids with greater nitrogen content increase food conversion efficiencies in larvae (Moeser and Vidal 2004b). Plant age affects survivorship, growth, and development of larvae—older plants are less suitable hosts than younger plants (Chege et al. 2005). Davis et al. (1996) suggested that the rate at which corn develops can lead to differences in eclosion, larval development, and beetle emergence. Larvae hatching during the first half of the eclosion period experience less mortality than those hatching during the second half (Oloumi-Sadeghi and Levine 1989). Root tissue from senescing corn plants is not suitable for larval development (Olmer and Hibbard 2008).

While corn is the predominant host for the western corn rootworm, larval survival and beetle emergence have been documented for a number of species in the family Poaceae (Branson and Ortman 1970; Clark and Hibbard 2004; Oyediran et al. 2004; Wilson and Hibbard 2004; Spencer and Raghu 2009). A list of species other than corn that can serve as potential hosts for larvae can be found in Table 1.1. However, these species differ in their suitability for larval development or beetle emergence. For many species other than corn, larval development is negatively affected and mortality is increased. However, Moeser and Vidal (2004a) observed that larval development was similar for corn when compared with a number of alternate hosts from Poaceae. Branson and Ortman (1967) noted that fertility was not affected when beetles emerged from green foxtail, *Setaria viridis* (L.) P. Beauv.; yellow foxtail, *S. leucopila* (Scribn. & Merr.) K. Schum.; Minter wheat, *Triticum aestivum* L.; Omugi barley, *Hordeum vulgare* L.; and intermediate wheatgrass, *Thinopyrum intermedium* Host. After going through three instars, larvae pupate in the soil (Steffey and Gray 2009).

Adults

Emergence of western corn rootworm beetles begins in late June and early July (Ball 1957). The number of beetles emerging from a cornfield is correlated with the number of eggs laid during the previous growing season (Weiss and Mayo 1985). Predicting seasonal emergence can be done reliably with a cumulative degree day model using atmospheric temperature as an input (Nowatzki et al. 2002). The first day beetles are observed in a field provides a good index for predicting cumulative emergence (Ruppel et al. 1978). Approximately 75% of emergence is completed by the R1 growth stage (silking) (Stevenson et al. 2008). Males begin to emerge approximately one week prior to females (Short and Hill 1972). Spatially, beetles have an aggregated pattern of emergence (Ellsbury et al. 1998) and distribution throughout a cornfield (Steffey and Tollefson 1982; Park and Tollefson 2006a). Under field conditions, beetles typically live for approximately 44 to 55 d (Ball 1957). However, under optimal conditions, longevity increases and can approach 100 d (Branson and Johnson 1973; Hill 1975). Longevity is influenced by timing of emergence—beetles emerging earlier during the growing season live longer than those emerging later (Boetel and Fuller 1997).

There are a number of compounds released by corn that are attractive to western corn rootworm beetles. Volatiles released by silks, especially senescing silks, elicit electroantennogram activity in beetles (Abou-Fakhr et al. 1996; Hibbard et al. 1997a). However, electroantennogram activity does not necessarily correlate to a behavioral response by beetles in the field (Hammack et al. 1999). Indole is a potent attractant for beetles (Andersen and Metcalf 1986). However, because indole is a food-derived attractant, it is not highly attractive to satiated beetles. Other compounds that are attractive include geranylacetone, linalool, methyl salicylate, and α-terpineol (Hammack 1996; Hammack 1997). In addition to attractants, corn produces a number of compounds that are phagostimulatory. Some amino acid phagostimulants include β-alanine, L-alanine, and L-serine (Kim and Mullin 1998). Pollen produces a novel phagostimulant, isorhamnetin 3-*O*-neohesperidoside (Kim and Mullin 2007). Cucurbitacins, bitter substances derived from plants in the family Cucurbitaceae, are phagostimulatory for many diabroticite species, including the western corn rootworm (Metcalf et al. 1980). Cucurbitacins are not attractive to beetles—they are considered feeding stimulants and/or arrestants (Howe et al. 1976). Historically, volatiles from cucurbits may have acted as synomones for beetles, providing a mechanism for pollination in these plants (Metcalf

et al. 1998). Some of these phagostimulatory compounds have been identified (Hibbard et al. 1997b), including (*E*,*E*)-3,5-octadien-2-one from the buffalo gourd, *Cucurbita foetidissima* Kunth (Cossé and Baker 1999).

Immediately after emergence, western corn rootworm beetles feed on leaf surfaces and move to silks and tassels as they become available (Bryson et al. 1953; Ball 1957). When silks and tassels become desiccated, beetles can feed on kernels on the tip of the ear. Plant phenology is the main factor leading to non-corn habitat utilization by beetles (Campbell and Meinke 2006). The nutritional quality of food sources is important—fecundity is enhanced when beetles consume fresh silks and pollen (Elliott et al. 1990a). Davidson et al. (2007) classified different cornfields based on their yield potential; beetles were more abundant in high yield potential fields than in fields with a low yield potential. Darnell et al. (2000) observed strong directional movement of beetles toward pollinating corn.

Western corn rootworm beetles are most active when temperatures are near 25 °C (VanWoerkom et al. 1980). Low temperatures and darkness limit beetle activity (Isard et al. 2004). Approximately 85% of beetles move less than 10 m/d through corn during reproductive stages (Spencer et al. 2003). Beetles have a low flight pattern that is strongly influenced by wind—initiation of flight decreases as wind speed increases (VanWoerkom et al. 1983; Isard et al. 2004). Beetles struggle to control flight direction at wind speeds exceeding 1.5 m/s (VanWoerkom et al. 1983) and can be dispersed significant distances by weather fronts (Grant and Seevers 1989). Witowski et al. (1975) described flight by beetles as bimodal, with peaks in flight occurring between 2 to 3 h after sunrise and before sunset; temperatures during these periods ranged from 22 to 27 °C. In addition to weather, age and sex influence movement as well. Males do not disperse as readily as females from their natal field (Hill and Mayo 1980). Ovarian development in females is negatively correlated with flight activity (Coats et al. 1987). Female flight activity can be characterized as trivial or sustained (Coats et al. 1986). Trivial flights are shorter (occurring over several minutes) and slower (16 m/min) than sustained flights (can last an hour or more at speeds of 49 m/min).

Ball and Chaudhury (1973) first demonstrated the existence of a sex pheromone in western corn rootworm females. Guss (1976) isolated the pheromone and observed a peak in attractiveness to males aged 7 to 9 d; however, the pheromone remained attractive to males 38 to 49 d old. This sex pheromone was later identified as racemic 8-methyl-2-decanol propanoate (Guss et al. 1982). Peaks in male response to the pheromone occur between 08:30 and 12:30 h and between 15:30 and 18:30 h (Dobson and Teal 1986). Temperatures below 15 °C

suppress the male response. Females secrete this pheromone from cuticular pores located on the seventh abdominal segment (Lew and Ball 1978). A female calling behavior (extension of the terminal abdominal segments) facilitates the release of this pheromone (Hammack 1995).

Most mating between western corn rootworm beetles occurs during July and August (Ball 1957). Teneral females are often mated immediately after emergence. Multiple matings, or mating attempts, can often be observed between the same pair of beetles. Males often mate more than once throughout their lives (Branson et al. 1977; Kang and Krupke 2009b). While females can mate multiple times during their lives, they do not mate while actively ovipositing (Branson et al. 1977). Multiple mating for females likely occurs in response to previously unsuccessful mating attempts. Mated females are generally unattractive to males (Bartlett and Chiang 1977). Bartlett and Chiang (1977) suggested that females cease emitting their sex pheromone after successful mating. Heavier females are more attractive to males than lighter ones—indicating that mate selection is not entirely random (Kang and Krupke 2009a).

For the western corn rootworm, both the physical act of mating and insemination are required for oocytes to mature (Sherwood and Levine 1993). A male-limited esterase is passed from males to females during copulation (Ruud et al. 1988). Mating consists of three discrete steps: preliminary courtship, physical stimulation of the female by the male, and copulation (Lew and Ball 1979). Physical stimulation occurs when the male taps his antennae against the female in an effort to pacify her. Maximal insemination of females can require copulation lasting 3 to 4 h (Lew and Ball 1980). After mating, the spermatophore degenerates after 7 d; spermatozoa can remain viable for up to 40 d.

Oviposition and fecundity in the western corn rootworm are significantly affected by female age (Elliott et al. 1990b). Females begin oviposition when they are approximately 20 d old (Short and Hill 1972). Hein and Tollefson (1985) used regression analysis to determine that oviposition is 50 and 90% complete by 19 August and 6 September, respectively. Optimum temperatures for oviposition occur between 15.6 to 18.3 °C (Ball 1957). Although females can lay in excess of 1000 eggs (Ball 1957), females rarely approach this number in the field (Elliott et al. 1990b). Oviposition under field conditions is likely well below its biotic potential (Hein and Tollefson 1987). Spatially, patterns of oviposition are aggregated (Hein et al. 1985b) and highly variable (Gray et al. 1992). While 80% of eggs are laid in the upper 15 cm of soil (Ball 1957), females can oviposit as deep as 30 to 35 cm

(Weiss et al. 1983; Gray et al. 1992). Females lay more eggs in the furrow (space between rows) than in the row (Weiss et al. 1983; Hein et al. 1985b). Females prefer to lay eggs at covered sites when compared with exposed sites (Guss et al. 1976). Females also prefer moist soil to dry soil for oviposition (Gustin 1979). Females may lay a significant portion of their eggs at sites where feeding reduces fitness (e.g., a non-host crop) (Siegfried and Mullin 1990). Additionally, elevated ozone concentrations stimulate oviposition (Schroeder et al. 2006).

Rotation-Resistance

Historically, oviposition by western corn rootworm females occurred predominantly in corn (Levine et al. 2002). As a result, rotating corn with a non-host crop was a widely successful management tactic due to the annual life cycle of this insect pest. Crops that can be rotated with corn to successfully prevent damage include oats, *Avena sativa* L.; sorghum, *Sorghum bicolor* (L.) Moench; soybean, *Glycine max* (L.) Merr.; and wheat (Bryson et al. 1953). Crop rotation remained effective since it was first recommended in the early 1900s (Gillette 1912). While this tactic continues to be efficacious for much of the range of this pest, some populations have evolved resistance to crop rotation (Levine et al. 2002). Severe root injury to first-year corn was observed in Illinois (Piper City, Ford County) in 1987 (Levine and Oloumi-Sadeghi 1996). As late as 1989, root injury to first-year corn throughout most of Illinois remained at subeconomic levels (Steffey et al. 1992). By 1995, however, crop rotation failed as a management tactic in a number of counties in east-central Illinois and northwestern Indiana (Levine et al. 2002). According to Gray et al. (2009), the current range of the rotation-resistant western corn rootworm includes the northern two-thirds of Illinois and Indiana, as well as portions of eastern Missouri and Iowa; southern Wisconsin and Michigan; and western Ohio. Rotation-resistance has been reported in southeastern Ontario as well (Knight et al. 2005).

Since the discovery of rotation-resistance in the western corn rootworm, a number of experiments have been performed to understand its underlying mechanism. Prolonged diapause has been eliminated as a potential explanation (Levine and Oloumi-Sadeghi 1996). Soybean is an acceptable site for oviposition in east-central Illinois (O'Neal et al. 1999). In fact, Pierce and Gray (2006a) determined that oviposition by rotation-resistant females was greater in soybean than in corn. The authors noted that females lay eggs in these two crops simultaneously, indicating that oviposition in soybean is not a response to declining quality of corn. In east-central Illinois, Rondon

and Gray (2003) observed densities of females large enough to cause damage to first-year corn following soybean; alfalfa, *Medicago sativa* L.; and potentially oat stubble. Beetle emergence has been recorded in corn following soybean and winter wheat throughout Illinois (Schroeder et al. 2005). Rondon and Gray (2004) attributed rotation-resistance to a lack of ovipositional fidelity to corn.

While rotation-resistant western corn rootworm females oviposit in a number of different crops, soybean is the most abundant non-corn crop found throughout much of the Midwest. Rotation-resistant beetles are not attracted to soybean (Spencer et al. 1999; O'Neal et al. 2004). Willingness of beetles to feed in soybean does not indicate a greater capacity to benefit nutritionally from soybean foliage (Mabry and Spencer 2003). Diet quality, as well as reproductive status, influences the likelihood of stress-induced oviposition. Exposure to a diet of soybean foliage increases the rate of oviposition, lowers the threshold for feeding, and increases the activity of beetles (Mabry et al. 2004). Fecundity is similar for rotation-resistant females receiving a corn-only diet or an alternating corn-soybean diet (Mabry et al. 2004). Oviposition by rotation-susceptible females is more likely to be affected by soybean herbivory when compared with rotation-resistant females (Knolhoff et al. 2010). This finding supports the suggestion by Mabry et al. (2004) that rotation-resistant females are less adversely affected by feeding on soybean tissue.

To date, the use of genetic techniques to differentiate between rotation-resistant and rotation-susceptible beetles has been unsuccessful (Miller et al. 2006). However, Garabagi et al. (2008) noted that expression of cyclic GMP-dependent protein kinases is 25% greater in rotation-resistant females than in rotation-susceptible females. Genes for these proteins have been implicated in the regulation of behavior in many animals. Curzi et al. (2012) reported that digestive enzymes play a role in facilitating rotation-resistance. The authors noted that rotation-resistant females had increased constitutive expression of cathepsin L-like proteases, allowing them to overcome soybean defenses (cysteine protease inhibitors) and forage for a longer duration in soybean than rotation-susceptible females.

Pierce and Gray (2006b) suggested that rotation-resistance was facilitated by decades of rigid annual crop rotation of corn and soybean. Rotation-resistance may have been caused by a single gene for beetle movement (Onstad et al. 2001b). One model for the expansion of the rotation-resistance trait predicts its spread at 10 to 30 km/year (Onstad et al. 1999). The rapid expansion of rotation-resistant western corn rootworm beetles was

promoted by high altitude flight, which Isard (2004) measured at approximately 10 m and noted was strongly correlated with predictable, diel changes in atmospheric conditions. Onstad et al. (2003a) hypothesized that as the amount of non-corn, non-rotated soybean in a geographical region increases, the expansion of rotation-resistance decreases. Successful strategies for slowing the spread of rotation-resistance include rotating soybean with rootworm-resistant transgenic corn or adopting a three-year rotation of corn, soybean, and wheat (Onstad et al. 2003b).

Injury and Damage

For the purposes of this dissertation, injury and damage will be discussed according to the definitions provided by Pedigo (2002), where *injury* is defined as "the effect of pest activities on host physiology that is usually deleterious" (e.g., loss of root tissue due to western corn rootworm larval feeding) and *damage* is defined as "a measurable loss of host utility" (e.g., yield loss). Once western corn rootworm larvae enter roots, they tunnel toward the root tip (Strnad and Bergman 1987). Plant height, root weight, root injury, and yield can be significantly correlated when evaluating larval feeding (Owens et al. 1974). Photosynthetic rates are often reduced when larvae begin feeding on roots (Godfrey et al. 1993; Riedell and Reese 1999; Urías-López et al. 2000). However, the degree of reduction and subsequent compensation may depend on plant phenology (Godfrey et al. 1993) or differences between hybrids (Urías-López et al. 2000). Root injury also limits utilization of soil moisture (Godfrey et al. 1993) and nitrogen (Spike and Tollefson 1989a). Additionally, root injury can lead to colonization by phytopathogenic or saprophytic fungi (Bryson et al. 1953; Kurtz et al. 2010). Environmental factors play a role in determining the severity of root injury. Excessive rainfall and saturated soil may prevent significant root injury (Spike and Tollefson 1988) due to increased larval mortality. Compacted soil results in less root injury as well (Ellsbury et al. 1994).

Corn plants can compensate for root injury caused by western corn rootworm larvae. Plants injured by larvae experience increased growth of adventitious roots (Riedell and Reese 1999). Hills and Peters (1971) developed a rating scale for root injury that accounts for root regrowth. Compensatory regrowth of roots negatively affects yield when soil moisture is adequate but positively affects yield when soil moisture is limiting (Gray and Steffey 1998). Compensatory regrowth is enhanced by nitrogen (Spike and Tollefson 1989b).

The accurate prediction of root injury by western corn rootworm larvae has been a goal of many researchers. Egg densities and subsequent root injury are not highly correlated (Tollefson 1990). Densities smaller than 1970 eggs/m of row may fail to result in a significant yield difference between corn treated with an insecticide and untreated corn (Sutter et al. 1990). Significant root injury occurs at densities near 3940 eggs/m of row (Branson et al. 1980). For a given egg density, western corn rootworm larvae produce greater root injury than northern corn rootworm larvae (Fisher 1985). If root injury is severe because of high larval densities, larval development is slowed and beetle emergence is reduced (Weiss et al. 1985). Using baited traps to monitor populations of females during August provides a reliable predictor for root injury during the subsequent year (Levine and Gray 1994); however, this sampling strategy has never been used widely by farmers.

In addition to the physiological symptoms associated with root injury caused by western corn rootworm larvae, root lodging can cause harvestability problems. Severe lodging can reduce yield by 11 to 34% (Spike and Tollefson 1991). Lodged plants are shorter and experience reduced light interception. Yield loss due to root injury or lodging is highly variable and influenced by a number of biotic and abiotic factors (Gray and Steffey 1998). Severity of yield loss due to lodging is related to soil moisture—dry soil conditions increase yield loss and can even result in plant death (Bryson et al. 1953; Spike and Tollefson 1989a). Yield loss is more subtly affected by root injury or lodging under conditions of minor to moderate environmental stress (Cox et al. 2008). When environmental conditions are optimal for corn development, yield loss due to root injury is highly variable (Spike and Tollefson 1989b).

A number of methods have been evaluated for estimating damage from root injury caused by western corn rootworm larvae. Root-injury ratings are more sensitive for predicting damage than estimates of vertical pulling weight, lodging, stunting, or beetle emergence (Branson et al. 1980). Evaluating root injury for corn grown under field conditions is more valuable for predicting damage than a greenhouse setting (Knutson et al. 1999). Oleson et al. (2005) developed a node-injury scale to assess root injury. The scale is linear (0 to 3) and based on the proportion of nodal roots that exhibit injury. One complete node of roots destroyed can result in yield reductions of 38, 480, and 1400 kg/ha under low, medium, and high stress conditions (Oleson et al. 2005). Depending on growing conditions, farmers can expect a 15% yield loss for each node of roots destroyed (Tinsley et al. 2013).

In addition to root injury caused by larvae, western corn rootworm beetles can injure corn plants as well. Beetles feed on most of the aboveground tissues, with the exception of the rigid stalk (Ball 1957). Beetle injury depends on a number of environmental factors (Culy et al. 1992). Moisture or heat stress magnify the effects of beetle injury, and an abundance of pollen can prevent injury to leaves, silks, and ears. While Culy et al. (1992) suggested that densities of 1 to 3 beetles/ear can reduce yield parameters due to silk feeding, Capinera et al. (1986) observed that densities as great as 20 beetles/ear do not significantly reduce yield. Compensation for beetle injury includes partitioning nutrients to uninjured portions of the ear (Capinera et al. 1986).

Management

Sampling

Sampling densities of western corn rootworm eggs, larvae, or beetles is an important component for both experimentation and management. Soil samples for eggs and larvae should be obtained at intervals of no less than 1.0 and 0.4 m, respectively (Park and Tollefson 2006b). Flotation is a superior method for sampling larvae when compared with visual searching or Berlese funnels (Bergman et al. 1981). When sampling beetles in cornfields, whole-plant counts are precise and cost-effective (Steffey et al. 1982). However, when using whole-plant counts, inter-observer variability should be considered (Shufran and Raney 1989). This variability can be eliminated by using sticky traps (Hein and Tollefson 1984). One important consideration when using sticky traps to sample insects is trap color. For western corn rootworm beetles, yellow is more attractive than other colors (Ball 1982) and is used for the popular Pherocon AM sticky trap (Great Lakes IPM, Vestaburg, MI). Sticky traps should be changed every 6 to 10 d to maintain reliable estimates (Hein and Tollefson 1984) and should be located at least 30 m apart to obtain spatial independence (Midgarden et al. 1993; Darnell et al. 1999). Lures can be used in conjunction with sticky traps (Hoffmann et al. 1996; Whitworth et al. 2002). Experimental sampling is often more intensive than sampling to guide management decisions. For example, labor-intensive single-plant emergence cages can be used to estimate beetle emergence (Hein et al. 1985a). Additionally, beetles can be tracked using isotopic (Nowatzki et al. 2003) or fluorescent (Toepfer et al. 2005b) markers—beetle detection depends on the persistence of the technique being used.

With regard to integrated pest management, an economic threshold is the number of insects that warrants a control measure to prevent increasing numbers of insects from reaching the economic injury level (i.e., the number of insects that will cause yield loss equivalent to the cost of the control measure) (Stern et al. 1959). Economic thresholds are variable and depend on a number of biotic (e.g., crop response to injury) and abiotic (e.g., environmental stress, crop value) factors. Steffey and Gray (2009) outlined the recommended scouting procedures for the western corn rootworm. Management decisions for the following year can be made by assessing beetle densities during late July through mid-August of the current year. The economic threshold for continuous corn is different than the economic threshold for corn grown after soybean. For continuous corn, approximately 50 whole-plant samples should be taken weekly. An average of 0.75 beetles/plant warrants a preventative management tactic (e.g., soil-applied insecticide) during the following year. For corn grown after soybean, 12 sticky traps should be spaced evenly throughout the soybean field where corn will be grown. Traps should be changed weekly, and an average of 5 to 10 beetles/trap per day warrants a preventative management tactic during the next growing season.

Agronomic Practices

Two agronomic practices that influence the western corn rootworm are tillage and weed management. Tillage affects the western corn rootworm in a number of ways. Gray and Tollefson (1987) noted that larval densities and root injury were less than expected for conservation tillage (e.g., no tillage) when compared with conventional tillage (e.g., fall plowing and spring cultivation). Conservation tillage reduces soil temperatures, which results in smaller root systems and a reduced amount of root tissue for larvae (Gray and Tollefson 1987). Additionally, conservation tillage delays beetle emergence when compared with conventional tillage (Pruess et al. 1968; Gray and Tollefson 1988a). Gray and Tollefson (1988a) observed a delayed initiation and increased rate of emergence for conservation tillage versus conventional tillage. However, the authors noted that cumulative emergence was similar for both tillage regimes.

Weed management also affects the western corn rootworm. A number of pollinating weed species are attractive to beetles (Hill and Mayo 1980). These species include smooth leaf pigweed, *Amaranthus hybridus* L.; rough pigweed, *A. retroflexus* L.; ragweed, *Ambrosia* spp.; foxtail, *Sertaria* spp.; and sorghum, *Sorghum* spp. Pollen

from weed species is exploited more frequently by beetles as corn matures and in weedier cornfields than in fields that are relatively weed-free (Moeser and Vidal 2005). Clark and Hibbard (2004) suggested that weeds that could potentially support larval development will become an important factor as the adoption of genetically modified corn hybrids (e.g., Bt corn) continues to increase. Larval development on weed species could negatively affect an integrated resistance management (IRM) program if larvae initially feed on weeds and subsequently move to Bt corn roots. Larval survival on weed species can negatively affect fitness. For example, beetles emerging from cornfields heavily infested with yellow foxtail are fewer in number and have smaller head capsules than beetles emerging from weed-free fields (Ellsbury et al. 2005).

Host Plant Resistance

The use of host plant resistance has been explored as a potential management tactic for the western corn rootworm. Species within the grass tribe Andropogoneae differ widely in their ability to support larvae (Branson 1971). Potential resistance to larvae has been documented in corn germplasm from Central America (Ortman and Branson 1976). While evaluating corn hybrids for resistance to the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), Gill et al. (2011) reported the existence of defense compounds with activity against western corn rootworm larvae that were induced by fall armyworm feeding. The corn hybrid SUM2162 has demonstrated antixenosis resistance to larvae (Bernklau et al. 2010). This hybrid prevents root injury (Bernklau et al. 2010) and reduces larval head capsule width and body weight (El Khishen et al. 2009). While evaluating corn hybrids from the 1980s, Riedell and Evenson (1993) suggested that large root systems may provide tolerance against feeding by western corn rootworm larvae; however, yield loss was substantial for these hybrids when root injury was severe. Due to the widespread success of insecticides and Bt corn for preventing damage caused by larvae, traditional host plant resistance is not used commonly.

Biological Control

Biological control for managing the western corn rootworm has been studied extensively. Entomopathogenic fungi can significantly and negatively affect larvae and beetles. Applications of the naturally occurring, soil-borne fungus *Beauveria bassiana* (Balsamo) Vuillemin cause significant beetle mortality (Mulock and Chandler 2000) and reduce fecundity (Mulock and Chandler 2001). While infection rates of emerging beetles are low, *B. bassiana* is

widespread (Bruck and Lewis 2001). Timing applications of this fungus to coincide with pollen shed increases infection when compared with applications coinciding with whorl stage corn (Bruck and Lewis 2002). Another fungal pathogen that causes significant mortality in larvae and beetles is *Metarhizium anisopliae* (Metchnikoff) Sorokin (Pilz et al. 2007). Beetles are more susceptible to *B. bassiana* and *M. anisopliae* than larvae.

The role of insects for biological control of the western corn rootworm is limited. In general, the predator community exploiting larvae as prey is restricted due to a hemolymph defense (Lundgren 2010). Lundgren et al. (2009) demonstrated that larval hemolymph is both sticky and chemically deterrent, which work in conjunction to deter predatory insects like *Poecilus cupreus* L. and *Harpalus pennsylvanicus* DeGeer (Coleoptera: Carabidae). Many of the generalist predators commonly found in cornfields do not exploit larvae or beetles as prey (Kuhlmann and van der Burgt 1998). There is a strong association between infestations of the cornfield ant, *Lasius neoniger* Emery, and reduced larval densities (Kirk 1981). Since cornfield ants do not feed on eggs (Ballard and Mayo 1979), they are most likely a predator of larvae moving toward corn plants (Kirk 1981). Lundgren and Fergen (2010) suggested that winter cover crops may positively influence the predator community associated with western corn rootworm larvae, resulting in higher rates of predator-induced larval mortality—especially among third instar larvae—and reduced root injury. Parasitoids rarely infect beetles in the field (Kuhlmann and van der Burgt 1998); however, parasitism rates by the tachinid *Celatoria compressa* Wulp can approach 27% in the laboratory (Zhang et al. 2004). This tachinid has been proposed as a classical biological control agent in Europe (Toepfer et al. 2009).

Entomopathogenic nematodes can be used to enhance biological control of the western corn rootworm (Kurtz et al. 2007). Nematodes from the genera *Heterorhabditis* and *Steinernema* have activity against larvae. Additionally, nematodes in these genera are unlikely to negatively affect nontarget species (Georgis et al. 1991). Larval mortality caused by some steinernematids approaches 77% in the laboratory (Toepfer et al. 2005a). Beetle emergence and root injury can be reduced by *S. carpocapsae* Weiser (Nickle et al. 1994; Journey and Ostlie 2000) and *H. bacteriophora* Poinar (Jackson 1996; Toepfer et al. 2010b). However, reducing beetle emergence with nematodes does not always result in decreased root injury (Toepfer et al. 2008). Some experiments have compared the efficacy of nematodes with insecticides. Wright et al. (1993) observed similar levels of control between *S. carpocapsae* and the organophosphates chlorpyrifos and terbufos. Pilz et al. (2009) reported similar results when comparing *H. bacteriophora* with the neonicotinoid clothianidin and the pyrethroid tefluthrin.

Nematodes can be applied effectively at planting using streamed-liquid or granular formulations (Toepfer et al. 2010a). However, experiments with nematodes using North American corn hybrids may produce different results compared with those performed using European hybrids. North American hybrids do not produce (*E*)-*β*-caryophyllene in response to root injury (Rasmann et al. 2005). This compound, produced by European hybrids, is attractive to entomopathogenic nematodes.

Insecticides

Throughout its history as a pest of corn, insecticides have played a critical role in managing the western corn rootworm. Organochlorines (a.k.a., chlorinated hydrocarbons) were widely used during the mid-1900s (Metcalf 2005). Examples include aldrin, dichlorodiphenyltrichloromethane (DDT), chlordane, heptachlor, and lindane. Applications of organochlorines result in significant larval (Burkhardt 1954a) and beetle (Burkhardt 1954b) mortality. However, beginning in the early 1970s, the use of these insecticides has diminished greatly because many are persistent environmental pollutants (Metcalf 2005). Currently, insecticides commonly used to prevent root injury include organophosphates, pyrethroids, and neonicotinoids.

Soil insecticides targeting western corn rootworm larvae are applied while planting. Post-planting rescue applications of insecticides are ineffective (Mayo 1976). Insecticides should be evaluated under different environmental conditions before determining an optimal rate of application (Musick and Fairchild 1968). In some cases, a reduced rate of insecticide (i.e., lower than the rate prescribed on the insecticide label) provides protection from root injury that is as effective as the full rate (Gray et al. 1993; Fuller et al. 1997; Boetel et al. 1998). Factors influencing efficacy of reduced rates include location in the Midwest, insecticide used, larval density, and environmental conditions, particularly levels of soil moisture (Fuller et al. 1997).

Gray et al. (2006) listed a number of explanations for performance problems with soil-applied insecticides: early planting (Musick and Fairchild 1967; Mayo 1980), poor calibration (Levine and Oloumi-Sadeghi 1991), dry soil conditions (Sutter et al. 1989), and lack of insecticide incorporation (Levine and Oloumi-Sadeghi 1991). Additionally, younger larvae are more susceptible to soil-applied insecticides than older larvae (Sutter 1982). While seed-applied insecticides are primarily used on modern corn hybrids to prevent injury from secondary pests like

wireworm, Elateridae spp., or grape colaspis, *Colaspis brunnea* (Fabricius), larvae (Gray 2011), they may protect against a limited amount of root injury caused by western corn rootworm larvae (Gray et al. 2006; Cox et al. 2007).

Insecticides targeting western corn rootworm beetles can be applied to corn foliage using aircraft or high-clearance tractor sprayers. Aerial application of malathion (an organophosphate) can reduce beetle densities by 39 to 72% (Pruess et al. 1974). Efficacy of foliar-applied insecticides is reduced by post-application rainfall or irrigation (Mayo 1984). Using a phagostimulant in conjunction with an insecticide has been suggested as a tactic for managing both larvae and beetles. For larvae, using a phagostimulant with thiamethoxam (a neonicotinoid) reduces the concentration of insecticide required for 50% mortality (LC_{50}) (Bernklau and Bjostad 2005). For beetles, cucurbitacin baits can be used to enhance insecticide efficacy (Metcalf et al. 1987). Reductions in beetle densities as great as 94% were achieved by combining carbaryl (a carbamate) with a cucurbitacin bait (Lance and Sutter 1990). However, prolonged use of phagostimulant-baited insecticides can lead to decreased susceptibility to the insecticide and reduced responsiveness to the bait (Siegfried et al. 2004).

Insecticide Resistance

The western corn rootworm has resistance or reduced susceptibility to several carbamate, organophosphate, and organochlorine insecticides. Chio and Metcalf (1979) used aldrin, carbofuran, fonofos, phorate, and terbufos to identify modes of resistance used by this insect—the authors identified epoxidation, hydroxylation, desulfuration, thioether oxidation, and hydrolysis. In 1962, LC_{50} values for aldrin and heptachlor (organochlorines) were 100-fold greater for beetles in Nebraska where insecticide applications were common when compared with areas where insecticide applications were used less frequently (Ball and Weekman 1962). Soon thereafter, Hamilton (1965) reported resistance to aldrin in portions of Iowa, Kansas, Minnesota, Missouri, and South Dakota. Resistance to aldrin is observed in both larvae and beetles (Hamilton 1966).

Overuse of various carbamates and organophosphates led to resistance to these insecticidal classes in some populations of the western corn rootworm (Call et al. 1977). Ball (1968) reported LC_{50} values for diazinon and phorate (organophosphates) nearly doubled from 1963 to 1967 in Nebraska. Additionally, some populations in Nebraska are resistant to carbaryl (a carbamate) and methyl parathion (an organophosphate) (Meinke et al. 1998). Resistance to carbaryl and methyl parathion is mediated by esterase-based hydrolysis and cytochrome P450-based

oxidation (Scharf et al. 1999a; Scharf et al. 1999b; Scharf et al. 2001; Zhou et al. 2004). Control failures associated with carbofuran (a carbamate) targeting larvae are exacerbated by enhanced degradation of the insecticide by adapted microbial populations (Felsot et al. 1982). Like aldrin, resistance to methyl parathion is observed in both larvae and beetles (Wright et al. 2000). Wright et al. (2000) noted that larval resistance to methyl parathion was likely due to selection pressure applied to adults—no insecticides targeting western corn rootworm larvae contained this active ingredient at the time of their experiment.

Bt Corn

The management of several key insect pests of corn changed in 1996 with the commercial introduction of genetically modified corn hybrids that express insecticidal proteins derived from the soil-borne bacterium *Bacillus thuringiensis* Berliner (Gill et al. 1992). A variety of *Bacillus thuringiensis* (Bt) strains naturally produce insecticidal crystal proteins, known as δ-endotoxins. These proteins are produced as protoxins, ingested by the insect, and solubilized in the midgut to produce an active toxin. Binding of the active toxin to the tissue lining the midgut results in perforations and subsequent death (Gill et al. 1992). Genes encoding for insecticidal toxins from *B. thuringiensis* are often modified or enhanced to increase their efficacy prior to transformation (Vaughn et al. 2005; Walters et al. 2008). While the first Bt hybrids primarily targeted the European corn borer, *Ostrinia nubilalis* Hübner, Bt hybrids targeting the western corn rootworm were commercialized in 2003 (Cry3Bb1, Event MON-ØØ863-5, YieldGard RW, Monsanto Co., St. Louis, MO), 2006 (Cry34/35Ab1, Event DAS-59122-7, Herculex RW, DuPont Pioneer, Johnston, IA, and Dow AgroSciences LLC, Indianapolis, IN), and 2007 (mCry3Aa, Event SYN-IR6Ø4-5, Agrisure RW, Syngenta, Basel, Switzerland) (Head and Ward 2009). These commercialized toxins have been described in great detail (Moellenbeck et al. 2001; Ellis et al. 2002; Schnepf et al. 2005; Vaughn et al. 2005; Raybould et al. 2007).

A concern related to the use of Bt corn is the effect that these toxins may have on nontarget species. Toxins expressed by Bt corn do not persist in the environment (Icoz and Stotzky 2008)—time until 50% degradation can be as little as two days (Herman et al. 2002). Bt toxins are not likely to serve as food allergens (Herman et al. 2003) and have no effect when ingested by hens (Scheideler et al. 2008) or rats (He et al. 2008; Healy et al. 2008). Likewise, toxins expressed by Bt corn pose no threat to soil decomposers (Hönemann et al. 2008), soil

microarthropods (Al-Deeb et al. 2003), or many of the beneficial insects found in cornfields (Lundgren and Wiedenmann 2002; Al-Deeb and Wilde 2003; Mullin et al. 2005).

When western corn rootworm larvae feed on Bt corn roots, mortality can be significant (Storer et al. 2006; Hibbard et al. 2010a). Other beneficial effects include decreases in larval mobility (Clark et al. 2006), root injury (Prischmann et al. 2007; Hibbard et al. 2009), and beetle emergence (Al-Deeb and Wilde 2005; Hibbard et al. 2009). Effects of Bt toxins on fitness estimators (e.g., larval or adult head capsule width) are varied (Hibbard et al. 2010a; Hibbard et al. 2011; Meihls et al. 2011; Murphy et al. 2011) and may interact with factors like density-dependent competition or timing of exposure to Bt roots. However, unlike Bt toxins targeting the European corn borer, Bt toxins targeting the western corn rootworm are not considered to be "high-dose" (Siegfried et al. 2005). As a result, some larval survival, beetle emergence, and root injury can be expected for Bt hybrids targeting the western corn rootworm (Gray and Steffey 2005). Larvae can survive on Bt corn by feeding on areas of the root where they may encounter a smaller dose of the Bt toxin (Al-Deeb and Wilde 2005). One factor affecting susceptibility to Bt corn is larval development—older larvae are less susceptible to Bt toxins than younger larvae (Binning et al. 2010). If larvae are able to establish on roots of Bt corn, larval growth and development proceed relatively normally (Hibbard et al. 2009).

Wilson et al. (2005) surveyed farmers regarding their opinions about Bt corn. Concerns cited most frequently were increased technology fees and decreased ability to market their harvested grain. Common benefits identified by farmers included reduced personal exposure to insecticide and decreased amounts of insecticide being introduced into the environment. Adoption of Bt corn has increased dramatically since its commercialization (USDA ERS 2012). Likewise, the number of Bt hybrids available to farmers that target the western corn rootworm has increased. For the most part, Bt hybrids provide acceptable control of this insect (Tinsley et al. 2010). However, the different toxins expressed in Bt corn can vary in their ability to prevent root injury. Additionally, the ability of a Bt toxin to prevent root injury can vary significantly between hybrids (Gray et al. 2007) or over time (Vaughn et al. 2005). Some of the newest Bt hybrids, known as "pyramided" hybrids, express more than one protein targeting western corn rootworm larvae (e.g., Genuity SmartStax, Monsanto Co.).

One of the principal considerations for managing the western corn rootworm with Bt corn is delaying the evolution of resistance to this technology. Some factors influencing the development of resistance to Bt corn

include inheritance of resistance, toxin dose, and refuge size/configuration (Onstad et al. 2001a). Fitness costs associated with resistance to Bt corn can delay the evolution of resistance by selecting against Bt-resistant larvae when they are not exposed to Bt corn (Gassmann et al. 2009). Additionally, using pyramided hybrids can help delay resistance because larvae are exposed to multiple Bt toxins with different modes of action (Baum et al. 2004; Onstad and Meinke 2010). Planting Bt corn is compatible with scouting and using economic thresholds (Gray 2000), a tactic that may help delay the evolution of resistance to Bt corn (Crowder et al. 2006).

Refuges for Bt corn help delay resistance by generating a large supply of susceptible beetles that are available to mate with any potentially resistant beetles emerging from Bt corn (Tabashnik et al. 2003). Single toxin Bt corn targeting the western corn rootworm requires a 20% refuge to be planted adjacent to or within the field containing the Bt hybrid (DiFonzo and Cullen 2011). Corn hybrids expressing multiple Bt toxins targeting this pest require a smaller refuge (5%) (DiFonzo and Cullen 2011). Gray et al. (2011) outlined the increasingly complicated refuge requirements for planting Bt corn to manage the western corn rootworm. As a result of confusion, refuge compliance may suffer (Jaffe 2009). Gray (2011) surveyed farmers in Illinois about their refuge practices—compliance ranged from 75 to 82%. Conversely, Andow et al. (2010) reported very low levels of compliance (only 2%) in southwestern Minnesota. Noncompliance was largely due to refuge blocks or strips that were too narrow.

Seed blends are being marketed as a way to reduce confusion and increase refuge compliance. Seed blends involve planting a Bt hybrid and non-Bt refuge hybrid that are premixed into a single bag of seed (Onstad et al. 2011). While seed blends may make pest monitoring more difficult (Onstad et al. 2011), they are as effective as block refuges for delaying resistance (Pan et al. 2011) and result in increased synchronization between beetles emerging from Bt corn and refuge corn (Murphy et al. 2010). The percentage of refuge corn for seed blends varies (5 to 10%) and depends on the hybrids being used (DiFonzo and Cullen 2011). Gassmann et al. (2011) reported the first confirmation of field-evolved resistance to Bt corn by the western corn rootworm. Severe root injury and lodging was reported in fields that contained hybrids expressing the Bt toxin Cry3Bb1 in 2009. Survival on corn with Cry3Bb1 was approximately 3-times greater for larvae from problem fields than for larvae from fields without control failures. The authors did not observe any evidence of cross-resistance for the Bt toxin Cry34/35Ab1. More recently, Gray (2012) reported that resistance to Cry3Bb1 has been confirmed in Illinois. In light of the recent

reports of resistance to Cry3Bb1, Tabashnik and Gould (2012) recommended that refuge requirements for single toxin and pyramided Bt hybrids should be increased to 50 and 20%, respectively.

References

Abou-Fakhr EM, Hibbard BE, Jewett DK, Bjostad LB, 1996. Electroantennogram responses of western corn rootworm (Coleoptera: Chrysomelidae) adults in relation to maize silk morphology and phenology. Environ. Entomol. 25, 430–435.

Al-Deeb MA, Wilde GE, 2003. Effect of *Bt* corn expressing the Cry3Bb1 toxin for corn rootworm control on aboveground nontarget arthropods. Environ. Entomol. 32, 1164–1170.

Al-Deeb MA, Wilde GE, 2005. Effect of *Bt* corn expressing the Cry3Bb1 toxin on western corn rootworm (Coleoptera: Chrysomelidae) biology. J. Kansas Entomol. Soc. 78, 142–152.

Al-Deeb MA, Wilde GE, Blair JM, Todd TC, 2003. Effect of *Bt* corn for corn rootworm control on nontarget soil microarthropods and nematodes. Environ. Entomol. 32, 859–865.

Andersen JF, Metcalf RL, 1986. Identification of a volatile attractant for *Diabrotica* and *Acalymma* spp. from blossoms of *Cucurbita maxima* Duchesne. J. Chem. Ecol. 12, 687–699.

Andow DA, Farrell SL, Hu Y, 2010. Planting patterns of in-field refuges observed for Bt maize in Minnesota. J. Econ. Entomol. 103, 1394–1399.

Ball HJ, 1957. On the biology and egg-laying habits of the western corn rootworm. J. Econ. Entomol. 50, 126–128.

Ball HJ, 1968. A five-year study of potential western corn rootworm resistance to diazinon and phorate in Nebraska. J. Econ. Entomol. 61, 496–498.

Ball HJ, 1982. Spectral response of the adult western corn rootworm (Coleoptera: Chrysomelidae) to selected wavelengths. J. Econ. Entomol. 75, 932–933.

Ball HJ, Chaudhury MFB, 1973. A sex attractant of the western corn rootworm. J. Econ. Entomol. 66, 1051–1053.

Ball HJ, Weekman GT, 1962. Insecticide resistance in the adult western corn rootworm in Nebraska. J. Econ. Entomol. 55, 439–441.

Ballard JB, Mayo ZB, 1979. Predatory potential of selected ant species on eggs of western corn rootworm. Environ. Entomol. 8, 575–576.

Bartlett RJ, Chiang HC, 1977. Field studies involving the sex-attractant pheromones of the western and northern corn rootworm beetles. Environ. Entomol. 6, 853–861.

Baum JA, Chu C, Rupar M, Brown GR, Donovan WP, Huesing JE, Ilagan O, Malvar TM, Pleau M, Walters M, Vaughn T, 2004. Binary toxins from *Bacillus thuringiensis* active against the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Appl. Environ. Microb. 70, 4889–4898.

Becker SC, Meinke LJ, 2008. A technique to morphologically differentiate larvae of *Diabrotica virgifera virgifera* and *D. barberi* (Coleoptera: Chrysomelidae). J. Kansas Entomol. Soc. 81, 77–79.

Berger HK, 2001. The western corn rootworm (*Diabrotica virgifera virgifera*): a new maize pest threatening Europe. Bull. OEPP 31, 411–414.

Bergman MK, Tollefson JJ, Hinz PN, 1981. Sampling scheme for estimating populations of corn rootworm larvae. Environ. Entomol. 10, 986–990.

Bernklau EJ, Bjostad LB, 1998a. Behavioral responses of first-instar western corn rootworm (Coleoptera: Chrysomelidae) to carbon dioxide in a glass bead bioassay. J. Econ. Entomol. 91, 444–456.

Bernklau EJ, Bjostad LB, 1998b. Reinvestigation of host location by western corn rootworm larvae (Coleoptera: Chrysomelidae): CO_2 is the only volatile attractant. J. Econ. Entomol. 91, 1331–1340.

Bernklau EJ, Bjostad LB, 2005. Insecticide enhancement with feeding stimulants in corn for western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98, 1150–1156.

Bernklau EJ, Bjostad LB, Meihls LN, Coudron TA, Lim E, Hibbard BE, 2009. Localized search cues in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. J. Econ. Entomol. 102, 558–562.

Bernklau EJ, Hibbard BE, Bjostad LB, 2010. Antixenosis in maize reduces feeding by western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 103, 2052–2060.

Binning RR, Lefko SA, Millsap AY, Thompson SD, Nowatzki TM, 2010. Estimating western corn rootworm (Coleoptera: Chrysomelidae) larval susceptibility to event DAS-59122-7 maize. J. Appl. Entomol. 134, 551–561.

Bjostad LB, Hibbard BE, 1992. 6-methyl-2-benzoxazolinone: a semiochemical for host location by western corn rootworm larvae. J. Chem. Ecol. 18, 931–944.

Boetel MA, Fuller BW, 1997. Seasonal emergence-time effects on adult longevity, fecundity, and egg viability of northern and western corn rootworms (Coleoptera: Chrysomelidae). Environ. Entomol. 26, 1208–1212.

Boetel MA, Fuller BW, Chandler LD, Hovland DG, Evenson PD, 1998. Fecundity and egg viability of northern and western corn rootworm (Coleoptera: Chrysomelidae) beetles surviving labeled and reduced soil insecticide applications. J. Econ. Entomol. 91, 274–279.

Boriani M, Agosti M, Kiss J, Edwards CR, 2006. Sustainable management of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), in infested areas: experiences in Italy, Hungary and the USA. Bull. OEPP 36, 531–537.

Branson TF, 1971. Resistance in the grass tribe Maydeae to larvae of the western corn rootworm. Ann. Entomol. Soc. Am. 64, 861–863.

Branson TF, 1987. The contribution of prehatch and posthatch development to protandry in the chrysomelid, *D. virgifera virgifera*. Entomol. Exp. Appl. 43, 205–208.

Branson TF, 1989. Survival of starved neonate larvae of *D. virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). J. Kansas Entomol. Soc. 62, 521–523.

Branson TF, Guss PL, Jackson JJ, 1977. Mating frequency of the western corn rootworm. Ann. Entomol. Soc. Am. 70, 506–508.

Branson TF, Johnson RD, 1973. Adult western corn rootworms: oviposition, fecundity, and longevity in the laboratory. J. Econ. Entomol. 66, 417–418.

Branson TF, Ortman EE, 1967. Fertility of western corn rootworm reared as larvae on alternate hosts. J. Econ. Entomol. 60, 595.

Branson TF, Ortman EE, 1970. The host range of larvae of the western corn rootworm: further studies. J. Econ. Entomol. 63, 800–803.

Branson TF, Sutter GR, 1985. Influence of population density of immatures on size, longevity, and fecundity of adult *D. virgifera virgifera* (Coleoptera: Chrysomelidae). Environ. Entomol. 14, 687–690.

Branson TF, Sutter GR, Fisher JR, 1980. Plant response to stress induced by artificial infestations of western corn rootworm. Environ. Entomol. 9, 253–257.

Bruck DJ, Lewis LC, 2001. Adult *Diabrotica* spp. (Coleoptera: Chrysomelidae) infection at emergence with indigenous *Beauveria bassiana* (Deuteromycotina: Hyphomycetes). J. Invertebr. Pathol. 77, 288–289.

Bruck DJ, Lewis LC, 2002. Whorl and pollen-shed stage application of *Beauveria bassiana* for suppression of adult western corn rootworm. Entomol. Exp. Appl. 103, 161–169.

Bryson HR, Wilbur DA, Burkhardt CC, 1953. The western corn rootworm, *Diabrotica virgifera* Lec. in Kansas. J. Econ. Entomol. 46, 995–999.

Burkhardt CC, 1954a. Chemical control of the western corn rootworm in Kansas in 1953. J. Econ. Entomol. 47, 691–696.

Burkhardt CC, 1954b. Control of injury by western corn rootworm adults. J. Econ. Entomol. 47, 358–359.

Calkins CO, Kirk VM, 1969. Effect of winter precipitation and temperature on overwintering eggs of northern and western corn rootworm. J. Econ. Entomol. 62, 541–543.

Call DJ, Walgenbach DD, Sutter GR, 1977. Corn rootworm cholinesterase activity as influenced by species, sex, age, and cold storage. J. Econ. Entomol. 70, 533–535.

Campbell LA, Meinke LJ, 2006. Seasonality and adult habitat use by four *Diabrotica* species at prairie-corn interfaces. Environ. Entomol. 35, 922–936.

Capinera JL, Epsky ND, Thompson DC, 1986. Effects of adult western corn rootworm (Coleoptera: Chrysomelidae) ear feeding on irrigated field corn in Colorado. J. Econ. Entomol. 79, 1609–1612.

Chege PG, Clark TL, Hibbard BE, 2005. Alternate host phenology affects survivorship, growth, and development of western corn rootworm (Coleoptera: Chrysomelidae) larvae. Environ. Entomol. 34, 1441–1447.

Chiang HC, 1973. Bionomics of the northern and western corn rootworms. Annu. Rev. Entomol. 18, 47–72.

Chiang HC, 1974. Temperature effects on hatching of eggs of the western corn rootworm, *Diabrotica virgifera*: factual and theoretical interpretations. Entomol. Exp. Appl. 17, 149–156.

Chio H, Metcalf RL, 1979. Detoxification mechanisms for aldrin, carbofuran, fonofos, phorate, and terbufos in four species of diabroticites. J. Econ. Entomol. 72, 732–738.

Ciosi M, Miller NJ, Kim KS, Giordano R, Estoup A, Guillemaud T, 2008. Invasion of Europe by the western corn rootworm, *Diabrotica virgifera virgifera*: multiple transatlantic introductions with various reductions of genetic diversity. Mol. Ecol. 17, 3614–3627.

Clark TL, Hibbard BE, 2004. Comparison of nonmaize hosts to support western corn rootworm (Coleoptera: Chrysomelidae) larval biology. Environ. Entomol. 33, 681–689.

Clark PL, Vaughn TT, Meinke LJ, Molina-Ochoa J, Foster JE, 2006. *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behavior on transgenic maize (MON 863) and its isoline. J. Econ. Entomol. 99, 722–727.

Coats SA, Mutchmor JA, Tollefson JJ, 1987. Regulation of migratory flight by juvenile hormone mimic and inhibitor in the western corn rootworm (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 80, 697–708.

Coats SA, Tollefson JJ, Mutchmor JA, 1986. Study of migratory flight in the western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 15, 1–6.

Cossé AA, Baker TC, 1999. Electrophysiologically and behaviorally active buffalo gourd root powder for corn rootworm beetles. J. Chem. Ecol. 25, 51–66.

Cox WJ, Shields E, Cherney DJR, 2008. Western corn rootworm damage subtly affects corn growth under moderate environmental stress. Crop Sci. 48, 1164–1169.

Cox WJ, Shields E, Cherney DJR, Cherney JH, 2007. Seed-applied insecticides inconsistently affect corn forage in continuous corn. Agron. J. 99, 1640–1644.

Crowder DW, Onstad DW, Gray ME, 2006. Planting transgenic insecticidal corn based on economic thresholds: consequences for integrated pest management and insect resistance management. J. Econ. Entomol. 99, 899–907.

Culy MD, Edwards CR, Cornelius JR, 1992. Effect of silk feeding by western corn rootworm (Coleoptera: Chrysomelidae) on yield and quality of inbred corn in seed corn production fields. J. Econ. Entomol. 85, 2440–2446.

Curzi MJ, Zavala JA, Spencer JL, Seufferheld MJ, 2012. Abnormally high digestive enzyme activity and gene expression explain the contemporary evolution of a *Diabrotica* biotype able to feed on soybeans. Ecol. Evol. 2, 2005–2017.

Darnell SJ, Meinke LJ, Young LJ, 2000. Influence of corn phenology on adult western corn rootworm (Coleoptera: Chrysomelidae) distribution. Environ. Entomol. 29, 587–595.

Darnell SJ, Meinke LJ, Young LJ, Gotway CA, 1999. Geostatistical investigation of the small-scale spatial variation of western corn rootworm (Coleoptera: Chrysomelidae) adults. Environ. Entomol. 28, 266–274.

Davidson SA, Peairs FB, Khosla R, 2007. Insect pest densities across site-specific management zones of irrigated corn in northeastern Colorado. J. Econ. Entomol. 100, 781–789.

Davis PM, Brenes N, Allee LL, 1996. Temperature dependent models to predict regional differences in corn rootworm (Coleoptera: Chrysomelidae) phenology Environ. Entomol. 25, 767–775.

Davis CS, Ni X, Quisenberry SS, Foster JE, 2000. Identification and quantification of hydroxamic acids in maize seedling root tissue and impact on western corn rootworm (Coleoptera: Chrysomelidae) larval development. J. Econ. Entomol. 93, 989–992.

DiFonzo C, Cullen E, 2011. Handy Bt Trait Table. Michigan State University Extension, East Lansing, Michigan.

Dobson ID, Teal PEA, 1986. Field studies of the temporal response patterns of male *Diabrotica virgifera virgifera* LeConte and *D. barberi* Smith and Lawrence to 8*R*-methyl-2*R*-decyl propanoate. Physiol. Entomol. 11, 405–410.

Dun Z, Mitchell PD, Agosti M, 2010. Estimating *Diabrotica virgifera virgifera* damage functions with field trial data: applying an unbalanced nested error component model. J. Appl. Entomol. 134, 409–419.

El Khishen AA, Bohn MO, Prischmann-Voldseth DA, Dashiell KE, French BW, Hibbard BE, 2009. Native resistance to western corn rootworm (Coleoptera: Chrysomelidae) larval feeding: characterization and mechanisms. J. Econ. Entomol. 102, 2350–2359.

Elliott NC, Gustin RD, Hanson SL, 1990a. Influence of adult diet on the reproductive biology and survival of the western corn rootworm, *Diabrotica virgifera virgifera*. Entomol. Exp. Appl. 56, 15–21.

Elliott NC, Lance DR, Hanson SL, 1990b. Quantitative description of the influence of fluctuating temperatures on the reproductive biology and survival of the western corn rootworm, *D. virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). Can. Entomol. 122, 59–68.

Ellis RT, Stockhoff BA, Stamp L, Schnepf HE, Schwab GE, Knuth M, Russell J, Cardineau GA, Narva KE, 2002. Novel *Bacillus thuringiensis* insecticidal crystal proteins active on western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Appl. Environ. Microbiol. 68, 1137–1145.

Ellsbury MM, Banken KR, Clay SA, Forcella F, 2005. Interactions among western corn rootworm (Coleoptera: Chrysomelidae), yellow foxtail, and corn. Environ. Entomol. 34, 627–634.

Ellsbury MM, Schumacher TE, Gustin RD, Woodson WD, 1994. Soil compaction effect on corn rootworm populations in maize artificially infested with eggs of western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 23, 943–948.

Ellsbury MM, Woodson WD, Clay SA, Malo D, Schumacher J, Clay DE, Carlson CG, 1998. Geostatistical characterization of the spatial distribution of adult corn rootworm (Coleoptera: Chrysomelidae) emergence. Environ. Entomol. 27, 910–917.

Felsot AS, Wilson JG, Kuhlman DE, Steffey KL, 1982. Rapid dissipation of carbofuran as a limiting factor in corn rootworm (Coleoptera: Chrysomelidae) control in fields with histories of continuous carbofuran use. J. Econ. Entomol. 75, 1098–1103.

Fisher JR, 1985. Comparison of controlled infestations of *Diabrotica virgifera virgifera* and *Diabrotica barberi* (Coleoptera: Chrysomelidae) on corn. J. Econ. Entomol. 78, 1406–1408.

Fisher JR, 1986. Development and survival of pupae of *D. virgifera virgifera* and *D. undecimpunctata howardi* (Coleoptera: Chrysomelidae) at constant temperatures and humidities. Environ. Entomol. 15, 626–630.

Fisher JR, 1989. Hatch of *D. virgifera virgifera* (Coleoptera: Chrysomelidae) eggs exposed to two different overwintering and hatch regimes. J. Kansas Entomol. Soc. 62, 607–610.

Fuller BW, Boetel MA, Walgenbach DD, Grundler JA, Hein GL, Jarvi KJ, Keaster AJ, Landis DA, Meinke LJ, Oleson JD, Ostlie KR, Tollefson JJ, Wedberg JL, Wilde GE, Evenson PD, 1997. Optimization of soil insecticide rates for managing corn rootworm (Coleoptera: Chrysomelidae) larvae in the north central United States. J. Econ. Entomol. 90, 1332–1340.

Garabagi F, French BW, Schaafsma AW, Pauls KP, 2008. Increased expression of a cGMP-dependent protein kinase in rotation-adapted western corn rootworm (*Diabrotica virgifera virgifera* L.). Insect Biochem. Molec. 38, 697–704.

Gassmann AJ, Carrière Y, Tabashnik BE, 2009. Fitness costs of insect resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 54, 147–163.

Gassmann AJ, Petzold-Maxwell JL, Keweshan RS, Dunbar MW, 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLOS ONE 6, E22629.

Georgis R, Kaya HK, Gaugler R, 1991. Effect of steinernematid and heterorhabditid nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on nontarget arthropods. Environ. Entomol. 20, 815–822.

Gill SS, Cowles EA, Pietrantonio PV, 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Annu. Rev. Entomol. 37, 615–636.

Gill TA, Sandoya G, Williams P, Luthe DS, 2011. Belowground resistance to western corn rootworm in lepidopteran-resistant maize genotypes. J. Econ. Entomol. 104, 299–307.

Gillette CP, 1912. *Diabrotica virgifera* Lec. as a corn root-worm. J. Econ. Entomol. 5, 364–366.

Godfrey LD, Meinke LJ, Wright RJ, 1993. Effects of larval injury by western corn rootworm (Coleoptera: Chrysomelidae) on gas exchange parameters of field corn. J. Econ. Entomol. 86, 1546–1556.

Godfrey LD, Meinke LJ, Wright RJ, Hein GL, 1995. Environmental and edaphic effects on western corn rootworm (Coleoptera: Chrysomelidae) overwintering egg survival. J. Econ. Entomol. 88, 1445–1454.

Grant RH, Seevers KP, 1989. Local and long-range movement of adult western corn rootworm (Coleoptera: Chrysomelidae) as evidenced by washup along southern Lake Michigan shores. Environ. Entomol. 18, 266–272.

Gray ME, 2000. Prescriptive use of transgenic hybrids for corn rootworms: an ominous cloud on the horizon? In: Proceedings of the 2000 Illinois Crop Protection Technology Conference. University of Illinois Extension, Urbana, IL, pp. 97–103.

Gray ME, 2011. Relevance of traditional integrated pest management (IPM) strategies for commercial corn producers in a transgenic agroecosystem: a bygone era? J. Agric. Food Chem. 59, 5852–5858.

Gray ME, 2012. Continuing evolution confirmed of field resistance to Cry3Bb1 in some Illinois fields by western corn rootworm. In: The Bulletin: Pest Management and Crop Development Information for Illinois, Issue No. 20. University of Illinois Extension, Urbana, IL, p. 120.

Gray ME, Estes RE, Tinsley NA, 2011. 0%, 5%, 10%, or 20%: stacked or pyramided, structured or refuge-in-a-bag—perfectly clear? In: Proceedings of the 2011 Corn and Soybean Classics, University of Illinois Extension, Urbana, IL, pp. 7–16.

Gray ME, Felsot AS, Steffey KL, Levine E, 1992. Planting time application of soil insecticides and western corn rootworm (Coleoptera: Chrysomelidae) emergence: implications for long-term management programs. J. Econ. Entomol. 85, 544–553.

Gray ME, Sappington TW, Miller NJ, Moeser J, Bohn MO, 2009. Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54, 303–321.

Gray ME, Steffey KL, 1998. Corn rootworm (Coleoptera: Chrysomelidae) larval injury and root compensation of 12 maize hybrids: an assessment of the economic injury index. J. Econ. Entomol. 91, 723–740.

Gray ME, Steffey KL, 2005. Transgenic YieldGard rootworm hybrid stumbles in Urbana experiment: why? In: Proceedings of the 2005 Illinois Crop Protection Technology Conference. University of Illinois Extension, Urbana, IL, pp. 41–46.

Gray ME, Steffey KL, Estes RE, Schroeder JB, 2007. Responses of transgenic maize hybrids to variant western corn rootworm larval injury. J. Appl. Entomol. 131, 386–390.

Gray ME, Steffey KL, Estes RE, Schroeder JB, Bakken DM, 2006. Transgenic corn rootworm hybrids, soil insecticides, and seed treatments: does anything work on the variant western corn rootworm? In: Proceedings of the 2006 Illinois Crop Protection Technology Conference. University of Illinois Extension, Urbana, IL, pp. 54–61.

Gray ME, Steffey KL, Oloumi-Sadeghi H, 1993. Participatory on-farm research in Illinois cornfields: an evaluation of established soil insecticide rates and prevalence of corn rootworm (Coleoptera: Chrysomelidae) injury. J. Econ. Entomol. 86, 1473–1482.

Gray ME, Tollefson JJ, 1987. Influence of tillage and western and northern corn rootworm (Coleoptera: Chrysomelidae) egg populations on larval populations and root damage. J. Econ. Entomol. 80, 911–915.

Gray ME, Tollefson JJ, 1988a. Emergence of the western and northern corn rootworms (Coleoptera: Chrysomelidae) from four tillage systems. J. Econ. Entomol. 81, 1398–1403.

Gray ME, Tollefson JJ, 1988b. Survival of the western and northern corn rootworms (Coleoptera: Chrysomelidae) in different tillage systems throughout the growing season of corn. J. Econ. Entomol. 81, 178–183.

Guss PL, 1976. The sex pheromone of the western corn rootworm (*Diabrotica virgifera*). Environ. Entomol. 5, 219–223.

Guss PL, Branson TF, Krysan JL, 1976. Adaptation of a dry diet for adults of the western corn rootworm. J. Econ. Entomol. 69, 503–505.

Guss PL, Tumlinson JH, Sonnet PE, Proveaux AT, 1982. Identification of a female-produced sex pheromone of the western corn rootworm. J. Chem. Ecol. 8, 545–556.

Gustin RD, 1979. Effect of two moisture and population levels on oviposition of the western corn rootworm. Environ. Entomol. 8, 406–407.

Gustin RD, 1981. Soil temperature environment of overwintering western corn rootworm eggs. Environ. Entomol. 10, 483–487.

Gustin RD, Schumacher TE, 1989. Relationship of some soil pore parameters to movement of first-instar western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 18, 343–346.

Hamilton EW, 1965. Aldrin resistance in corn rootworm beetles. J. Econ. Entomol. 58, 297–300.

Hamilton EW, 1966. LD_{50}s for aldrin against resistant larvae and adults of western corn rootworm. 59, 473–474.

Hammack L, 1995. Calling behavior in female western corn rootworm beetles (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 88, 562–569.

Hammack L, 1996. Corn volatiles as attractants for northern and western corn rootworm beetles (Coleoptera: Chrysomelidae: *Diabrotica* spp.). J. Chem. Ecol. 22, 1237–1253.

Hammack L, 1997. Attractiveness of synthetic corn volatiles to feral northern and western corn rootworm beetles (Coleoptera: Chrysomelidae). Environ. Entomol. 26, 311–317.

Hammack L, Hibbard BE, Holyoke CW, Kline M, Leva DM, 1999. Behavioral responses of corn rootworm adults to host plant volatiles perceived by western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 28, 961–967.

He XY, Huang KL, Li X, Qin W, Delaney B, Luo YB, 2008. Comparison of grain from corn rootworm resistant transgenic DAS-59122-7 maize with non-transgenic maize grain in a 90-day feeding study in Sprague-Dawley rats. Food Chem. Toxicol. 46, 1994–2002.

Head G, Ward D, 2009. Insect resistance in corn through biotechnology. In: Biotechnology in Agriculture and Forestry Volume 63: Molecular Genetic Approaches to Maize Improvement, ed. Kriz AL, Larkins BA. Springer-Verlag, Berlin, Germany, pp. 31–40.

Healy C, Hammond B, Kirkpatrick J, 2008. Results of a 13-week safety assurance study with rats fed grain from corn rootworm-protected, glyphosate-tolerant MON 88017 corn. Food Chem. Toxicol. 46, 2517–2524.

Hein GL, Bergman MK, Bruss RG, Tollefson JJ, 1985a. Absolute sampling technique for corn rootworm (Coleoptera: Chrysomelidae) adult emergence that adjusts to fit common row spacing. J. Econ. Entomol. 78, 1503–1506.

Hein GL, Tollefson JJ, 1984. Comparison of adult corn rootworm (Coleoptera: Chrysomelidae) trapping techniques as population estimators. Environ. Entomol. 13, 266–271.

Hein GL, Tollefson JJ, 1985. Seasonal oviposition of northern and western corn rootworms (Coleoptera: Chrysomelidae) in continuous cornfields. J. Econ. Entomol. 78, 1238–1241.

Hein GL, Tollefson JJ, 1987. Model of the biotic potential of western corn rootworm (Coleoptera: Chrysomelidae) adult populations, and its use in studying population dynamics. Environ. Entomol. 16, 446–452.

Hein GL, Tollefson JJ, Hinz PN, 1985b. Design and cost considerations in the sampling of northern and western corn rootworm (Coleoptera: Chrysomelidae) eggs. J. Econ. Entomol. 78, 1495–1499.

Herman RA, Schafer BW, Korjagin VA, Ernest AD, 2003. Rapid digestion of Cry34Ab1 and Cry35Ab1 in simulated gastric fluid. J. Agric. Food Chem. 51, 6823–6827.

Herman RA, Scherer PN, Wolt JD, 2002. Rapid degradation of a binary, PS149B1, *δ*-endotoxin of *Bacillus thuringiensis* in soil, and a novel mathematical model for fitting curve-linear decay. Environ. Entomol. 31, 208–214.

Hibbard BE, Bernklau EJ, Bjostad LB, 1994. Long-chain free fatty acids: semiochemicals for host location by western corn rootworm larvae. J. Chem. Ecol. 20, 3335–3344.

Hibbard BE, Clark TL, Ellersieck MR, Meihls LN, El Khishen AA, Kaster V, Steiner H, Kurtz R, 2010a. Mortality of western corn rootworm larvae on MIR604 transgenic maize roots: field survivorship has no significant impact on survivorship of F1 progeny on MIR604. J. Econ. Entomol. 103, 2187–2196.

Hibbard BE, El Khishen AA, Vaughn TT, 2009. Impact of MON863 transgenic roots is equivalent on western corn rootworm larvae for a wide range of maize phenologies. J. Econ. Entomol. 102, 1607–1613.

Hibbard BE, Frank DL, Kurtz R, Boudreau E, Ellersieck MR, Odhiambo JF, 2011. Mortality impact of Bt transgenic maize roots expressing eCry3.1Ab, mCry3A, and eCry3.1Ab plus mCry3A on western corn rootworm larvae in the field. J. Econ. Entomol. 104, 1584–1591.

Hibbard BE, Higdon ML, Duran DP, Schweikert YM, Ellersieck MR, 2004. Role of egg density on establishment and plant-to-plant movement by western corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 97, 871–882.

Hibbard BE, Meihls LN, Ellersieck MR, Onstad DW, 2010b. Density-dependent and density-independent mortality of the western corn rootworm: impact on dose calculations of rootworm-resistant Bt corn. J. Econ. Entomol. 103, 77–84.

Hibbard BE, Randolph TL, Bernklau EJ, Abou-Fakhr EM, Bjostad LB, 1997a. Electroantennogram-active components of maize silk for adults of the western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 26, 285–296.

Hibbard BE, Randolph TL, Bernklau EJ, Bjostad LB, 1997b. Electroantennogram-active components in buffalo gourd root powder for western corn rootworm adults (Coleoptera: Chrysomelidae). Environ. Entomol. 26, 1136–1142.

Hill RE, 1975. Mating, oviposition patterns, fecundity and longevity of the western corn rootworm. J. Econ. Entomol. 68, 311–315.

Hill RE, Mayo ZB, 1980. Distribution and abundance of corn rootworm species as influenced by topography and crop rotation in eastern Nebraska. Environ. Entomol. 9, 122–127.

Hills TM, Peters DC, 1971. A method of evaluating post-planting insecticide treatments for control of western corn rootworm larvae. J. Econ. Entomol. 64, 764–765.

Hoffmann MP, Kirkwyland JJ, Smith RF, Long RF, 1996. Field tests with kairomone-baited traps for cucumber beetles and corn rootworms in cucurbits. Environ. Entomol. 25, 1173–1181.

Hönemann L, Zurbrügg C, Nentwig W, 2008. Effects of *Bt*-corn decomposition on the composition of the soil meso- and macrofauna. Appl. Soil Ecol. 40, 203–209.

Howe WL, Sanborn JR, Rhodes AM, 1976. Western corn rootworm adult and spotted cucumber beetle associations with *Cucurbita* and cucurbitacins. Environ. Entomol. 5, 1043–1048.

Icoz I, Stotzky G, 2008. Cry3Bb1 protein from *Bacillus thuringiensis* in root exudates and biomass of transgenic corn does not persist in soil. Transgenic Res. 17, 609–620.

Isard SA, Spencer JL, Mabry TR, Levine E, 2004. Influence of atmospheric conditions on high-elevation flight of western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 33, 650–656.

Jackson JJ, 1996. Field performance of entomopathogenic nematodes for suppression of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 89, 366–372.

Jackson JJ, Elliott NC, 1988. Temperature-dependent development of immature stages of the western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Environ. Entomol. 17, 166–171.

Jaffe G, 2009. Complacency on the Farm: Significant Noncompliance with EPA's Refuge Requirements Threatens the Future Effectiveness of Genetically Engineered Pest-Protected Corn. Center for Science in the Public Interest, Washington DC.

Journey AM, Ostlie KR, 2000. Biological control of the western corn rootworm (Coleoptera: Chrysomelidae) using the entomopathogenic nematode, *Steinernema carpocapsae*. Environ. Entomol. 29, 822–831.

Kang J, Krupke CH, 2009a. Influence of weight of male and female western corn rootworm (Coleoptera: Chrysomelidae) on mating behaviors. Ann. Entomol. Soc. Am. 102, 326–332.

Kang J, Krupke CH, 2009b. Likelihood of multiple mating in *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). J. Econ. Entomol. 102, 2096–2100.

Kim JH, Mullin CA, 1998. Structure-phagostimulatory relationships for amino acids in adult western corn rootworm, *Diabrotica virgifera virgifera*. J. Chem. Ecol. 24, 1499–1511.

Kim JH, Mullin CA, 2007. An isorhamnetin rhamnoglycoside serves as a costimulant for sugars and amino acids in feeding responses of adult western corn rootworms (*Diabrotica virgifera virgifera*) to corn (*Zea mays*) pollen. J. Chem. Ecol. 33, 501–512.

Kirk VM, 1981. Corn rootworm: population reduction associated with the ant, *Lasius neoniger*. Environ. Entomol. 10, 966–967.

Kiss J, Edwards CR, Berger HK, Cate P, Cean M, Cheek S, Derron J, Festić H, Furlan L, Igrc Barčić J, Ivanova I, Lammers W, Omelyuta V , Princzinger G, Reynaud P, Sivcev I, Sivicek P, Urek G, Vahala O, 2005a. Monitoring of western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in Europe 1992–2003. In: Western Corn Rootworm: Ecology and Management, ed. Vidal S, Kuhlmann U, Edwards CR. CABI, Cambridge, United Kingdom, pp. 29–39.

Kiss J, Komáromi J, Bayar K, Edwards CR, Hatala-Zsellér I, 2005b. Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) and the crop rotation systems in Europe. In: Western Corn Rootworm: Ecology and Management, ed. Vidal S, Kuhlmann U, Edwards CR. CABI, Cambridge, United Kingdom, pp. 189–220.

Knight CC, Pauls KP, Sears MK, Schaafsma AW, 2005. Oviposition site selected by the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in southern Ontario strip plots. Can. J. Plant Sci. 85, 949–954.

Knolhoff LM, Glas JJ, Spencer JL, Berenbaum MR, 2010. Oviposition behaviors in relation to rotation resistance in the western corn rootworm. Environ. Entomol. 39, 1922–1928.

Knutson RJ, Hibbard BE, Barry BD, Smith VA, Darrah LL, 1999. Comparison of screening techniques for western corn rootworm (Coleoptera: Chrysomelidae) host-plant resistance. J. Econ. Entomol. 92, 714–722.

Krysan JL, 1978. Diapause, quiescence, and moisture in the egg of the western corn rootworm, *Diabrotica virgifera*. J. Insect Physiol. 24, 535–540.

Krysan JL, 1982. Diapause in the nearctic species of the *virgifera* group of *Diabrotica*: evidence for tropical origin and temperate adaptations. Ann. Entomol. Soc. Am. 75, 136–142.

Kuhlmann U, van der Burgt WACM, 1998. Possibilities for biological control of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in central Europe. Biocontrol News Info. 19, 59N–68N.

Kurtz B, Karlovsky P, Vidal S, 2010. Interaction between western corn rootworm (Coleoptera: Chrysomelidae) larvae and root-infecting *Fusarium verticillioides*. Environ. Entomol. 39, 1532–1538.

Kurtz B, Toepfer S, Ehlers RU, Kuhlmann U, 2007. Assessment of establishment and persistence of entomopathogenic nematodes for biological control of western corn rootworm. J. Appl. Entomol. 131, 420–425.

Lance DR, Sutter GR, 1990. Field-cage and laboratory evaluations of semiochemical-based baits for managing western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 83, 1085–1090.

Levine E, Gray ME, 1994. Use of cucurbitacin vial traps to predict corn rootworm (Coleoptera: Chrysomelidae) larval injury in a subsequent crop of corn. J. Entomol. Sci. 29, 590–600.

Levine E, Oloumi-Sadeghi H, 1991. Management of diabroticite rootworms in corn. Annu. Rev. Entomol. 36, 229–255.

Levine E, Oloumi-Sadeghi H, 1996. Western corn rootworm (Coleoptera: Chrysomelidae) larval injury to corn grown for seed production following soybeans grown for seed production. J. Econ. Entomol. 89, 1010–1016.

Levine E, Oloumi-Sadeghi H, Ellis CR, 1992. Thermal requirements, hatching patterns, and prolonged diapause in western corn rootworm (Coleoptera: Chrysomelidae) eggs. J. Econ. Entomol. 85, 2425–2432.

Levine E, Spencer JL, Isard SA, Onstad DW, Gray ME, 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Am. Entomol. 48, 94–107.

Lew AC, Ball HJ, 1978. The structure of the apparent pheromone-secreting cells in female *Diabrotica virgifera*. Ann. Entomol. Soc. Am. 71, 685–688.

Lew AC, Ball HJ, 1979. The mating behavior of the western corn rootworm *Diabrotica virgifera* (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 72, 391–393.

Lew AC, Ball HJ, 1980. Effect of copulation time on spermatozoa transfer of *Diabrotica virgifera* (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 73, 360–361.

Lundgren JG, Fergen JK, 2010. The effects of a winter cover crop on *Diabrotica virgifera* (Coleoptera: Chrysomelidae) populations and beneficial arthropod communities in no-till maize. Environ. Entomol. 39, 1816–1828.

Lundgren JG, Haye T, Toepfer S, Kuhlmann U, 2009. A multifaceted hemolymph defense against predation in *Diabrotica virgifera virgifera* larvae. Biocontrol Sci. Techn. 19, 871–880.

Lundgren JG, Toepfer S, Haye T, Kuhlmann U, 2010. Haemolymph defence of an invasive herbivore: its breadth of effectiveness against predators. J. Appl. Entomol. 134, 439–448.

Lundgren JG, Wiedenmann RN, 2002. Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a nontarget species, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae). Environ. Entomol. 31, 1213–1218.

Mabry TR, Spencer JL, 2003. Survival and oviposition of a western corn rootworm variant feeding on soybean. Entomol. Exp. Appl. 109, 113–121.

Mabry TR, Spencer JL, Levine E, Isard SA, 2004. Western corn rootworm (Coleoptera: Chrysomelidae) behavior is affected by alternating diets of corn and soybean. Environ. Entomol. 33, 860–871.

MacDonald PJ, Ellis CR, 1990. Survival time of unfed, first-instar western corn rootworm (Coleoptera: Chrysomelidae) and the effects of soil type, moisture, and compaction on their mobility in soil. Environ. Entomol. 19, 666–671.

Mangelsdorf PC, 1983. The mystery of corn: new perspectives. Proc. Am. Phil. Soc. 127, 215–247.

Mayo ZB, 1976. Emergency postplanting applications of insecticides to control larvae of the western and northern corn rootworm in Nebraska. J. Econ. Entomol. 69, 600–602.

Mayo ZB, 1980. Influence of planting dates on the efficacy of soil insecticides applied to control larvae of the western and northern corn rootworm. J. Econ. Entomol. 73, 211–212.

Mayo ZB, 1984. Influences of rainfall and sprinkler irrigation on the residual activity of insecticides applied to corn for control of adult western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 77, 190–193.

Meihls LN, Higdon ML, Ellersieck M, Hibbard BE, 2011. Selection for resistance to mCry3A-expressing transgenic corn in western corn rootworm. J. Econ. Entomol. 104, 1045–1054.

Meinke LJ, Siegfried BD, Wright RJ, Chandler LD, 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J. Econ. Entomol. 91, 594–600.

Melhus IE, Painter RH, Smith FO, 1954. A search for resistance to the injury caused by species of *Diabrotica* in the corns of Guatemala. Iowa State Coll. J. Sci. 29, 75–94.

Metcalf RL, 1986. Foreword. In: Methods for the Study of Pest *Diabrotica*, ed. Krysan JL, Miller TA. Springer, New York, NY, pp. vii–xv.

Metcalf RL, 2005. Insect control. In: Ullmann's Encyclopedia of Industrial Chemistry, ed. Bellussi G, Bohnet M, Bus J, Drauz K, Greim H, Jäckel K, Karst U, Kleemann A, Kreysa G, Laird T, Meier W, Ottow E, Röper M, Scholtz J, Sundmacher K, Ulber R, Wietelmann U. Wiley-VCH, Weinheim, Germany, pp. 1–64.

Metcalf RL, Ferguson JE, Lampman R, Anderson JF, 1987. Dry cucurbitacins-containing baits for controlling diabroticite beetles (Coleoptera: Chrysomelidae). J. Econ. Entomol. 80, 870–875.

Metcalf RL, Lampman RL, Lewis PA, 1998. Competitive kairomonal chemical ecology of diabroticite beetles (Coleoptera: Chrysomelidae: Galerucinae: Luperini: Diabroticina) in a reconstituted tallgrass prairie ecosystem. J. Econ. Entomol. 91, 881–890.

Metcalf RL, Metcalf RA, Rhodes AM, 1980. Cucurbitacins as kairomones for diabroticite beetles. Proc. Natl. Acad. Sci. USA 77, 3769–3772.

Midgarden DG, Youngman RR, Fleischer SJ, 1993. Spatial analysis of counts of western corn rootworm (Coleoptera: Chrysomelidae) adults on yellow sticky traps in corn: geostatistics and dispersion indices. Environ. Entomol. 22, 1124–1133.

Miller NJ, Kim KS, Ratcliffe ST, Estoup A, Bourguet D, Guillemaud T, 2006. Absence of genetic divergence between western corn rootworms (Coleoptera: Chrysomelidae) resistant and susceptible to control by crop rotation. J. Econ. Entomol. 99, 685–690.

Moellenbeck DJ, Peters ML, Bing JW, Rouse JR, Higgins LW, Sims L, Nevshemal T, Marshall L, Ellis RT, Bystrak PG, Lang BA, Stewart JL, Kouba K, Sondag V, Gustafson V, Nour K, Xu D, Swenson J, Zhang J, Czapla T, Schwab G, Jayne S, Stockhoff BA, Narva K, Schnepf HE, Stelman SJ, Poutre C, Koziel M, Duck N, 2001. Insecticidal proteins from *Bacillus thuringiensis* protect corn from corn rootworms. Nat. Biotechnol. 19, 668–672.

Moeser J, Vidal S, 2004a. Do alternative host plants enhance the invasion of the maize pest *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae, Galerucinae) in Europe? Environ. Entomol. 33, 1169–1177.

Moeser J, Vidal S, 2004b. Response of larvae of invasive maize pest *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to carbon/nitrogen ratio and phytosterol content of European maize varieties. J. Econ. Entomol. 97, 1335–1341.

Moeser J, Vidal S, 2005. Nutritional resources used by the invasive maize pest *Diabrotica virgifera virgifera* in its new south-east-European distribution range. Entomol. Exp. Appl. 114, 55–63.

Mullin CA, Saunders II MC, Leslie TW, Biddinger DJ, Fleischer SJ, 2005. Toxic and behavioral effects to Carabidae of seed treatments used on Cry3Bb1- and Cry1Ab/c-protected corn. Environ. Entomol. 34, 1626–1636.

Mulock B, Chandler L, 2000. Field-cage studies of *Beauveria bassiana* (Hyphomycetes: Moniliaceae) for the suppression of adult western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Biocontrol Sci. Techn. 10, 51–60.

Mulock BS, Chandler LD, 2001. Effect of *Beauveria bassiana* on the fecundity of western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Biol. Control 22, 16–21.

Murphy AF, Ginzel MD, Krupke CH, 2010. Evaluating western corn rootworm (Coleoptera: Chrysomelidae) emergence and root damage in a seed mix refuge. J. Econ. Entomol. 103, 147–157.

Murphy AF, Seiter NJ, Krupke CH, 2011. The impact of Bt maize as a natal host on adult head capsule width in field populations of western corn rootworm. Entomol. Exp. Appl. 139, 8–16.

Musick GJ, Fairchild ML, 1967. Preliminary study on some of the factors affecting control of western corn rootworm larvae with soil insecticides. J. Econ. Entomol. 60, 1522–1525.

Musick GJ, Fairchild ML, 1968. Comparison of application rates of some soil insecticides for control of western corn rootworm larvae in Missouri. J. Econ. Entomol. 61, 1188–1189.

Musick GJ, Fairchild ML, 1971. Field studies on rate of hatch of western corn rootworm eggs in Missouri during 1965–68. J. Econ. Entomol. 64, 9–11.

Nickle WR, Connick Jr. WJ, Cantelo WW, 1994. Effects of pesta-pelletized *Steinernema carpocapsae* (All) on western corn rootworms and Colorado potato beetles. J. Nematol. 26, 249–250.

Nowatzki TM, Niimi B, Warren KJ, Putnam S, Meinke LJ, Gosselin DC, Harvey FE, Hunt TE, Siegfried BD, 2003. In-field labeling of western corn rootworm adults (Coleoptera: Chrysomelidae) with rubidium. J. Econ. Entomol. 96, 1750–1759.

Nowatzki TM, Tollefson JJ, Calvin DD, 2002. Development and validation of models for predicting the seasonal emergence of corn rootworm (Coleoptera: Chrysomelidae) beetles in Iowa. Environ. Entomol. 31, 864–873.

Oleson JD, Park Y, Nowatzki TM, Tollefson JJ, 2005. Node-injury scale to evaluate root injury by corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98, 1–8.

Olmer KJ, Hibbard BE, 2008. The nutritive value of dying maize and *Setaria faberi* roots for western corn rootworm (Coleoptera: Chrysomelidae) development. J. Econ. Entomol. 101, 1547–1556.

Oloumi-Sadeghi H, Levine E, 1989. Effect of starvation and time of egg hatch on larval survival of the western corn rootworm, *D. virgifera virgifera* (Coleoptera: Chrysomelidae), in the laboratory. J. Kansas Entomol. Soc. 62, 108–116.

O'Neal ME, Gray ME, Smyth CA, 1999. Population characteristics of a western corn rootworm (Coleoptera: Chrysomelidae) strain in east-central Illinois corn and soybean fields. J. Econ. Entomol. 92, 1301–1310.

O'Neal ME, Landis DA, Miller JR, DiFonzo CD, 2004. Corn phenology influences *Diabrotica virgifera virgifera* emigration and visitation to soybean in laboratory assays. Environ. Entomol. 33, 35–44.

Onstad DW, Crowder DW, Isard SA, Levine E, Spencer JL, O'Neal ME, Ratcliffe ST, Gray ME, Bledsoe LW, DiFonzo CD, Eisley JB, Edwards CR, 2003a. Does landscape diversity slow the spread of rotation-resistant western corn rootworm (Coleoptera: Chrysomelidae)? Environ. Entomol. 32, 992–1001.

Onstad DW, Crowder DW, Mitchell PD, Guse CA, Spencer JL, Levine E, Gray ME, 2003b. Economics versus alleles: balancing integrated pest management and insect resistance management for rotation-resistant western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 96, 1872–1885.

Onstad DW, Guse CA, Spencer JL, Levine E, Gray ME, 2001a. Modeling the dynamics of adaptation to transgenic corn by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 94, 529–540.

Onstad DW, Hibbard BE, Clark TL, Crowder DW, Carter KG, 2006. Analysis of density-dependent survival of *Diabrotica* (Coleoptera: Chrysomelidae) in cornfields. Environ. Entomol. 35, 1272–1278.

Onstad DW, Joselyn MG, Isard SA, Levine E, Spencer JL, Bledsoe LW, Edwards CR, DiFonzo CS, Willson H, 1999. Modeling the spread of western corn rootworm (Coleoptera: Chrysomelidae) populations adapting to soybean-corn rotation. Environ. Entomol. 28, 188–194.

Onstad DW, Meinke LJ, 2010. Modeling evolution of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to transgenic corn with two insecticidal traits. J. Econ. Entomol. 103, 849–860.

Onstad DW, Mitchell PD, Hurley TM, Lundgren JG, Porter RP, Krupke CH, Spencer JL, DiFonzo CD, Baute TS, Hellmich RL, Buschman LL, Hutchison WD, Tooker JF, 2011. Seeds of change: corn seed mixtures for resistance management and integrated pest management. J. Econ. Entomol. 104, 343–352.

Onstad DW, Spencer JL, Guse CA, Levine E, Isard SA, 2001b. Modeling evolution of behavioral resistance by an insect to crop rotation. Entomol. Exp. Appl. 100, 195–201.

Ortman EE, Branson TF, 1976. Growth pouches for studies of host plant resistance to larvae of corn rootworms. J. Econ. Entomol. 69, 380–382.

Owens JC, Witkowski JF, Tollefson JJ, Rogers RR, Peters DC, 1974. Greenhouse evaluation of soil insecticides for western corn rootworm control. J. Econ. Entomol. 67, 772–774.

Oyediran IO, Hibbard BE, Clark TL, 2004. Prairie grasses as hosts of the western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 33, 740–747.

Palmer DF, French LK, Chiang HC, 1977. Effects of chill temperatures on developing western corn rootworm eggs. Environ. Entomol. 6, 862–864.

Pan Z, Onstad DW, Nowatzki TM, Stanley BH, Meinke LJ, Flexner JL, 2011. Western corn rootworm (Coleoptera: Chrysomelidae) dispersal and adaptation to single-toxin transgenic corn deployed with block or blended refuge. Environ. Entomol. 40, 964–978.

Park YL, Tollefson JJ, 2006a. Development and economic evaluation of spatial sampling plans for corn rootworm *Diabrotica* spp. (Col., Chrysomelidae) adults. J. Appl. Entomol. 130, 337–342.

Park YL, Tollefson JJ, 2006b. Spatial distributions of corn rootworm (Coleoptera: Chrysomelidae) eggs and larvae: implications for sampling. J. Kansas Entomol. Soc. 79, 129–135.

Pedigo LP, 2002. Economic decision levels for pest populations. In: Entomology and Pest Management. Pearson Education Inc., Upper Saddle River, NJ, pp. 255–287.

Piedrahita O, Ellis CR, Bogart JP, 1985. Interaction of northern and western corn rootworm larvae (Coleoptera: Chrysomelidae) in a controlled environment. Environ. Entomol. 14, 138–141.

Pierce CMF, Gray ME, 2006a. Seasonal oviposition of a western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), variant in east central Illinois commercial maize and soybean fields. Environ. Entomol. 35, 676–683.

Pierce CMF, Gray ME, 2006b. Western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), oviposition: a variant's response to maize phenology. Environ. Entomol. 35, 423–434.

Pilz C, Keller S, Kuhlmann U, Toepfer S, 2009. Comparative efficacy assessment of fungi, nematodes, and insecticides to control western corn rootworm larvae in maize. Biocontrol 54, 671–684.

Pilz C, Wegensteiner R, Keller S, 2007. Selection of entomopathogenic fungi for the control of western corn rootworm *Diabrotica virgifera virgifera*. J. Appl. Entomol. 131, 426–431.

Prischmann DA, Dashiell KE, Schneider DJ, Hibbard BE, 2007. Field screening maize germplasm for resistance and tolerance to western corn rootworms (Col.: Chrysomelidae). J. Appl. Entomol. 131, 406–415.

Pruess KP, Weekman GT, Somerhalder BR, 1968. Western corn rootworm egg distribution and adult emergence under two corn tillage systems. J. Econ. Entomol. 61, 1424–1427.

Pruess KP, Witkowski JF, Raun ES, 1974. Population suppression of western corn rootworm by adult control with ULV malathion. J. Econ. Entomol. 67, 651–655.

Rasmann S, Köllner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ, 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434, 732–737.

Raybould A, Stacey D, Vlachos D, Graser G, Li X, Joseph R, 2007. Non-target organism risk assessment of MIR604 maize expressing mCry3A for control of corn rootworm. J. Appl. Entomol. 131, 391–399.

Riedell WE, Evenson PD, 1993. Rootworm feeding tolerance in single-cross maize hybrids from different eras. Crop Sci. 33, 951–955.

Riedell WE, Reese RN, 1999. Maize morphology and shoot CO_2 assimilation after root damage by western corn rootworm larvae. Crop Sci. 39, 1332–1340.

Roehrdanz RL, 2003. Multiplex polymerase chain reaction method for differentiating western and northern corn rootworm larvae (Coleoptera: Chrysomelidae). J. Econ. Entomol. 96, 669–672.

Rondon SI, Gray ME, 2003. Captures of western corn rootworm (Coleoptera: Chrysomelidae) adults with Pherocon AM and vial traps in four crops in east central Illinois. J. Econ. Entomol. 96, 737–747.

Rondon SI, Gray ME, 2004. Ovarian development and ovipositional preference of the western corn rootworm (Coleoptera: Chrysomelidae) variant in east central Illinois. J. Econ. Entomol. 97, 390–396.

Ruppel RF, Russell HL, Jennings SJ, 1978. Indices for projecting emergence of corn rootworm adults in Michigan. J. Econ. Entomol. 71, 947–949.

Ruud SK, McDonald IC, Gabrielson DA, Johnson OA, 1988. Species distribution and characterization studies of sex-limited esterases within the *virgifera* group of *Diabrotica* (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 81, 954–963.

Schaafsma AW, Fuentes JD, Gillespie TJ, Whitfield GH, Ellis CR, 1993. Performance of a model for egg hatching of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, using measured and modeled soil temperatures as input. Int. J. Biometerol. 37, 11–18.

Scharf ME, Meinke LJ, Siegfried BD, Wright RJ, Chandler LD, 1999a. Carbaryl susceptibility, diagnostic concentration determination, and synergism for U.S. populations of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 92, 33–39.

Scharf ME, Meinke LJ, Wright RJ, Chandler LD, Siegfried BD, 1999b. Metabolism of carbaryl by insecticide-resistant and -susceptible western corn rootworm populations (Coleoptera: Chrysomelidae). Pestic. Biochem. and Physiol. 63, 85–96.

Scharf ME, Parimi S, Meinke LJ, Chandler LD, Siegfried BD, 2001. Expression and induction of three family 4 cytochrome P450 (CYP4) genes identified from insecticide-resistant and susceptible western corn rootworms, *Diabrotica virgifera virgifera*. Insect Mol. Biol. 10, 139–146.

Scheideler SE, Hileman RE, Weber T, Robeson L, Hartnell GF, 2008. The in vivo digestive fate of the Cry3Bb1 protein in laying hens fed diets containing MON 863 corn. Poultry Sci. 87, 1089–1097.

Schnepf HE, Lee S, Dojillo J, Burmeister P, Fencil K, Morera L, Nygaard L, Narva KE, Wolt JD, 2005. Characterization of Cry34/Cry35 binary insecticidal proteins from diverse *Bacillus thuringiensis* strain collections. Appl. Environ. Microb. 71, 1765–1774.

Schroeder JB, Gray ME, Ratcliffe ST, Estes RE, Long SP, 2006. Effects of elevated CO_2 on a variant of the western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 35, 637–644.

Schroeder JB, Ratcliffe ST, Gray ME, 2005. Effect of four cropping systems on variant western corn rootworm (Coleoptera: Chrysomelidae) adult and egg densities and subsequent larval injury in rotated maize. J. Econ. Entomol. 98, 1587–1593.

Schumann M, Vidal S, 2012. Dispersal and spatial distribution of western corn rootworm larvae in relation to root phenology. Agric. For. Entomol. 14, 331–339.

Sherwood DR, Levine E, 1993. Copulation and its duration affects female weight, oviposition, hatching patterns, and ovarian development in the western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 86, 1664–1671.

Short DE, Hill RE, 1972. Adult emergence, ovarian development, and oviposition sequence of the western corn rootworm in Nebraska. J. Econ. Entomol. 65, 685–689.

Short DE, Luedtke RJ, 1970. Larval migration of the western corn rootworm. J. Econ. Entomol. 63, 325–326.

Shufran KA, Raney HG, 1989. Influence of inter-observer variation on insect scouting observations and management decisions. J. Econ. Entomol. 82, 180–185.

Siegfried BD, Meinke LJ, Parimi S, Scharf ME, Nowatzki TJ, Zhou X, Chandler LD, 2004. Monitoring western corn rootworm (Coleoptera: Chrysomelidae) susceptibility to carbaryl and cucurbitacin baits in the areawide management pilot program. J. Econ. Entomol. 97, 1726–1733.

Siegfried BD, Mullin CA, 1990. Effects of alternative host plants on longevity, oviposition, and emergence of western and northern corn rootworms (Coleoptera: Chrysomelidae). Environ. Entomol. 19, 474–480.

Siegfried BD, Vaughn TT, Spencer T, 2005. Baseline susceptibility of western corn rootworm (Coleoptera: Chrysomelidae) to Cry3Bb1 *Bacillus thuringiensis* toxin. J. Econ. Entomol. 98, 1320–1324.

Smith RF, Lawrence JF, 1967. Clarification of the status of the type specimens of diabroticites. Univ. Calif. Publ. Entomol. 45, 174.

Spencer JL, Isard SA, Levine E, 1999. Free flight of western corn rootworm (Coleoptera: Chrysomelidae) to corn and soybean plants in a walk-in wind tunnel. J. Econ. Entomol. 92, 146–155.

Spencer JL, Mabry TR, Vaughn TT, 2003. Use of transgenic plants to measure insect herbivore movement. J. Econ. Entomol. 96, 1738–1749.

Spencer JL, Raghu S, 2009. Refuge or reservoir? The potential impacts of the biofuel crop *Miscanthus* × *giganteus* on a major pest of maize. PLOS ONE 4, E8336.

Spike BP, Tollefson JJ, 1988. Western corn rootworm (Coleoptera: Chrysomelidae) larval survival and damage potential to corn subjected to nitrogen and plant density treatments. J. Econ. Entomol. 81, 1450–1455.

Spike BP, Tollefson JJ, 1989a. Relationship of plant phenology to corn yield loss resulting from western corn rootworm (Coleoptera: Chrysomelidae) larval injury, nitrogen deficiency, and high plant density. J. Econ. Entomol. 82, 226–231.

Spike BP, Tollefson JJ, 1989b. Relationship of root ratings, root size, and root regrowth to yield of corn injured by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 82, 1760–1763.

Spike BP, Tollefson JJ, 1991. Yield response of corn subjected to western corn rootworm (Coleoptera: Chrysomelidae) infestation and lodging. J. Econ. Entomol. 84, 1585–1590.

Steffey KL, Gray ME, 2009. Managing insect pests. In: Illinois Agronomy Handbook. University of Illinois Extension, Urbana, IL, pp. 179–196.

Steffey KL, Gray ME, Kuhlman DE, 1992. Extent of corn rootworm (Coleoptera: Chrysomelidae) larval damage in corn after soybeans: search for the expression of the prolonged diapause trait in Illinois. J. Econ. Entomol. 85, 268–275.

Steffey KL, Tollefson JJ, 1982. Spatial dispersion patterns of northern and western corn rootworm adults in Iowa cornfields. Environ. Entomol. 11, 283–286.

Steffey KL, Tollefson JJ, Hinz PN, 1982. Sampling plan for population estimation of northern and western corn rootworm adults in Iowa cornfields. Environ. Entomol. 11, 287–291.

Stern VM, Smith RF, van den Bosch R, Hagen KS, 1959. The integrated control concept. Hilgardia 29, 81–101.

Stevenson DE, Michels GJ, Bible JB, Jackman JA, Harris MK, 2008. Physiological time model for predicting adult emergence of western corn rootworm (Coleoptera: Chrysomelidae) in the Texas High Plains. J. Econ. Entomol. 101, 1584–1593.

Storer NP, Babcock JM, Edwards JM, 2006. Field measures of western corn rootworm (Coleoptera: Chrysomelidae) mortality caused by Cry34/35Ab1 proteins expressed in maize event 59122 and implications for trait durability. J. Econ. Entomol. 99, 1381–1387.

Strnad SP, Bergman MK, 1987. Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. Environ. Entomol. 16, 1193–1198.

Strnad SP, Bergman MK, Fulton WC, 1986. First-instar western corn rootworm (Coleoptera: Chrysomelidae) response to carbon dioxide. Environ. Entomol. 15, 839–842.

Strnad SP, Dunn PE, 1990. Host search behaviour of neonate western corn rootworm (*Diabrotica virgifera virgifera*). J. Insect Physiol. 36, 201–205.

Sutter GR, 1982. Comparative toxicity of insecticides for corn rootworm (Coleoptera: Chrysomelidae) larvae in a soil bioassay. J. Econ. Entomol. 75, 489–491.

Sutter GR, Branson TF, Fisher JR, Elliott NC, Jackson JJ, 1989. Effect of insecticide treatments on root damage ratings of maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 82, 1792–1798.

Sutter GR, Fisher JR, Elliott NC, Branson TF, 1990. Effect of insecticide treatments on root lodging and yields of maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 83, 2414–2420.

Suttle PJ, Musick GJ, Fairchild ML, 1967. Study of larval migration of western corn rootworm. J. Econ. Entomol. 60, 1226–1228.

Tabashnik BE, Carrière Y, Dennehy TJ, Morin S, Sisterson MS, Roush RT, Shelton AM, Zhao J, 2003. Insect resistance to transgenic Bt crops: lessons from the laboratory and field. J. Econ. Entomol. 96, 1031–1038.

Tabashnik BE, Gould F, 2012. Delaying corn rootworm resistance to Bt corn. J. Econ. Entomol. 105, 767–776.

Tinsley NA, Estes RE, Gray ME, 2010. Evaluation of products to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2010. In: On Target: 2010 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 5–11.

Tinsley NA, Estes RE, Gray ME, 2013. Validation of a nested error component model to estimate damage caused by corn rootworm larvae. J. Appl. Entomol. 137, 161–169.

Toepfer S, Burger R, Ehlers R, Peters A, Kuhlmann U, 2010a. Controlling western corn rootworm larvae with entomopathogenic nematodes: effect of application techniques on plant-scale efficacy. J. Appl. Entomol. 134, 467–480.

Toepfer S, Gueldenzoph C, Ehlers R, Kuhlmann U, 2005a. Screening of entomopathogenic nematodes for virulence against the invasive western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in Europe. Bull. Entomol. Res. 95, 473–482.

Toepfer S, Hatala-Zseller I, Ehlers R, Peters A, Kuhlmann U, 2010b. The effect of application techniques on field-scale efficacy: can the use of entomopathogenic nematodes reduce damage by western corn rootworm larvae? Agric. For. Entomol. 12, 389–402.

Toepfer S, Kuhlmann U, 2004. Survey for natural enemies of the invasive alien chrysomelid, *Diabrotica virgifera virgifera*, in central Europe. Biocontrol 49, 385–395.

Toepfer S, Levay N, Kiss J, 2005b. Suitability of different fluorescent powders for mass-marking the chrysomelid, *Diabrotica virgifera virgifera* LeConte. J. Appl. Entomol. 129, 456–464.

Toepfer S, Peters A, Ehlers R, Kuhlmann U, 2008. Comparative assessment of the efficacy of entomopathogenic nematode species at reducing western corn rootworm larvae and root damage in maize. J. Appl. Entomol. 132, 337–348.

Toepfer S, Zhang F, Kuhlmann U, 2009. Assessing host specificity of a classical biological control agent against western corn rootworm with a recently developed testing protocol. Biol. Control 51, 26–33.

Tollefson JJ, 1990. Comparison of adult and egg sampling for predicting subsequent populations of western and northern corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 83, 574–579.

Turpin FT, Peters DC, 1971. Survival of southern and western corn rootworm larvae in relation to soil texture. J. Econ. Entomol. 64, 1448–1451.

Urías-López MA, Meinke LJ, Higley LG, Haile FJ, 2000. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on photosynthetic rate and vegetative growth of different types of maize. Environ. Entomol. 29, 861–867.

USDA ERS [United States Department of Agriculture Economic Research Service], 2012. Adoption of Genetically Engineered Crops in the US. United States Department of Agriculture, Washington DC.

USDA FAS [United States Department of Agriculture Foreign Agricultural Service], 2012. World Agricultural Production (12-12). United States Department of Agriculture, Washington DC.

VanWoerkom GJ, Turpin FT, Barrett Jr. JR, 1980. Influence of constant and changing temperatures on locomotor activity of adult western corn rootworms (*Diabrotica virgifera*) in the laboratory. Environ. Entomol. 9, 32–34.

VanWoerkom GJ, Turpin FT, Barrett Jr. JR, 1983. Wind effect on western corn rootworm (Coleoptera: Chrysomelidae) flight behavior. Environ. Entomol. 12, 196–200.

Vaughn T, Cavato T, Brar G, Coombe T, DeGooyer T, Ford S, Groth M, Howe A, Johnson S, Kolacz K, Pilcher C, Purcell J, Romano C, English L, Pershing J, 2005. A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. Crop Sci. 45, 931–938.

Walters FS, Stacy CM, Lee MK, Palekar N, Chen JS, 2008. An engineered chymotrypsin/cathepsin G site in domain I renders *Bacillus thuringiensis* Cry3A active against western corn rootworm larvae. Appl. Environ. Microb. 74, 367–374.

Weiss MJ, Mayo ZB, 1985. Influence of corn plant density on corn rootworm (Coleoptera: Chrysomelidae) population estimates. Environ. Entomol. 14, 701–704.

Weiss MJ, Mayo ZB, Newton JP, 1983. Influence of irrigation practices on the spatial distribution of corn rootworm (Coleoptera: Chrysomelidae) eggs in the soil. Environ. Entomol. 12, 1293–1295.

Weiss MJ, Seevers KP, Mayo ZB, 1985. Influence of western corn rootworm (*D. virgifera virgifera*) larval densities and damage on corn rootworm survival, developmental time, size and sex ratio (Coleoptera: Chrysomelidae). J. Kansas Entomol. Soc. 58, 397–402.

Wesseler J, Fall EH, 2010. Potential damage costs of *Diabrotica virgifera virgifera* infestation in Europe—the 'no control' scenario. J. Appl. Entomol. 134, 385–394.

Whitworth RJ, Wilde GE, Shufran RA, Milliken GA, 2002. Comparison of adult corn rootworm (Coleoptera: Chrysomelidae) sampling methods. J. Econ. Entomol. 95, 96–105.

Wilson TA, Hibbard BE, 2004. Host suitability of nonmaize agroecosystem grasses for the western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 33, 1102–1108.

Wilson TA, Rice ME, Tollefson JJ, Pilcher CD, 2005. Transgenic corn for control of the European corn borer and corn rootworms: a survey of Midwestern farmers' practices and perceptions. J. Econ. Entomol. 98, 237–247.

Witkowski JF, Owens JC, Tollefson JJ, 1975. Diel activity and vertical flight distribution of adult western corn rootworm in Iowa cornfields. J. Econ. Entomol. 68, 351–352.

Woodson WD, 1994. Interspecific and intraspecific larval competition between *Diabrotica virgifera virgifera* and *Diabrotica barberi* (Coleoptera: Chrysomelidae). Environ. Entomol. 23, 612–616.

Wright RJ, Scharf ME, Meinke LJ, Zhou X, Siegfried BD, Chandler LD, 2000. Larval susceptibility of an insecticide-resistant western corn rootworm (Coleoptera: Chrysomelidae) population to soil insecticides: laboratory bioassays, assays of detoxification enzymes, and field performance. J. Econ. Entomol. 93, 7–13.

Wright RJ, Witkowski JF, Echtenkamp G, Georgis R, 1993. Efficacy and persistence of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) applied through a center-pivot irrigation system against larval corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 86, 1348–1354.

Zhang F, Toepfer S, Riley K, Kuhlmann U, 2004. Reproductive biology of *Celatoria compressa* (Diptera: Tachinidae), a parasitoid of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Biocontrol Sci. Techn. 14, 5–16.

Zhou X, Scharf ME, Sarath G, Meinke LJ, Chandler LD, Siegfried BD, 2004. Partial purification and characterization of a methyl-parathion resistance-associated general esterase in *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Pestic. Biochem. Physiol. 78, 114–125.

Table 1.1 List of species in the family Poaceae other than corn, *Zea mays* (L.), that can support at least limited larval development of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte

Subfamily	Tribe	Species
Arundinoideae	Aristideae	Purple three awn, *Aristida purpurea* Nutt.[1,2]
Bambusoideae	Oryzeae	Rice, *Oryza sativa* L.[4]
Chloridoideae	Cynodonteae	Sideoats grama, *Bouteloua curtipendula* (Michx.) Torr.[1]
		Buffalograss, *Bouteloua dactyloides* (Nutt.) Columbus[1]
		Rhodesgrass, *Chloris gayana* Kunth[2,3]
		Windmillgrass, *Chloris verticillata* Nutt.[2]
		Bermudagrass, *Cynodon dactylon* (L.) Pers.[2]
		Galleta, *Pleuraphis jamesii* Torr.[1]
		Prairie cordgrass, *Spartina pectinata* Link[1]
	Eragrostideae	Weeping lovegrass, *Eragrostis curvula* (Schrad.) Nees[3,4]
		Sand lovegrass, *Eragrostis trichodes* Wood[1,3,4]
		Tall dropseed, *Sporobolus compositus* (Poir.) Merr.[1]
		Sand dropseed, *Sporobolus cryptandrus* (Torr.) A. Gray[1]
Panicoideae	Andropogoneae	Miscanthus, *Miscanthus* × *giganteus*[5]
		Little bluestem, *Schizachyrium scoparium* (Michx.) Nash[1]
		Indiangrass, *Sorghastrum nutans* (L.) Nash[1]
		Sorghum, *Sorghum bicolor* (L.) Moench[2]
	Paniceae	Large crabgrass, *Digitaria sanguinalis* (L.) Scop.[2,3]
		Barnyardgrass, *Echinochloa muricata* (P. Beauv.) Fernald[3]
		Woolly cupgrass, *Eriochloa villosa* (Thunb.) Kunth[3]
		Witchgrass, *Panicum capillare* L.[1]
		Fall panicum, *Panicum dichotomiflorum* Michx.[2]
		Proso millet, *Panicum miliaceum* L.[4]
		Switchgrass, *Panicum virgatum* L.[3]
		Dallisgrass, *Paspalum dilatatum* Poir.[2]
		Giant foxtail, *Setaria faberi* Herrm.[2,3]
		Yellow foxtail, *Setaria leucopila* (Scribn. & Merr.) K. Schum.[2–4]
		Bristly foxtail, *Setaria verticillata* (L.) P. Beauv.[3]

[1]Oyediran et al. 2004. [2]Wilson and Hibbard 2004. [3]Clark and Hibbard 2004. [4]Branson and Ortman 1970. [5]Spencer and Raghu 2009.

Table 1.1 (cont.) List of species in the family Poaceae other than corn, *Zea mays* (L.), that can support at least limited larval development of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte

Subfamily	Tribe	Species
Panicoideae (cont.)	Paniceae (cont.)	Green foxtail, *Setaria viridis* (L.) P. Beauv.[3,4]
		Texas panicum, *Urochloa texana* (Buckl.) R. Webster[3]
Pooideae	Aveneae	Redtop, *Agrostis gigantea* L.[3]
		Creeping bentgrass, *Agrostis stolonifera* L.[2]
		Reed canarygrass, *Phalaris arundinacea* L.[3]
	Bromeae	Smooth brome, *Bromus inermis* Leyss.[2]
	Poeae	Tall fescue, *Festuca arundinacea* Schreb.[2]
		Annual ryegrass, *Lolium multiflorum* Lam.[2]
		Annual bluegrass, *Poa annua* L.[2]
		Kentucky bluegrass, *Poa pratensis* L.[2]
	Stipeae	Needle-and-threadgrass, *Hesperostipa comata* (T. & R.) Bark.[1]
		New Mexico feathergrass, *Hesperostipa neomexicana* (Th.) Bark.[1]
		Porcupinegrass, *Hesperostipa spartea* (Trin.) Bark.[1]
		Green needlegrass, *Nasella viridula* (Trin.) Bark.[1]
	Triticeae	Jointed goatgrass, *Aegilops cylindrica* Host[2]
		Pubescent wheatgrass, *Agropyron trichohorum* Link[1,4]
		Canada wildrye, *Elymus canadensis* L.[1]
		Squirreltail, *Elymus elymoides* (Raf.) Swezey[1]
		Slender wheatgrass, *Elymus trachycaulus* (Link) Gould ex Shin.[1,4]
		Tall wheatgrass, *Elytrigia elongata* (Host) Nevski[4]
		Quackgrass, *Elytrigia repens* (L.) Desv. Ex Nevski[2]
		Little barley, *Hordeum pusillum* Nutt.[2]
		Barley, *Hordeum vulgare* L.[4]
		Western wheatgrass, *Pascopyrum smithii* (Rydb.) Á. Löve[1–4]
		Cereal rye, *Secale cereale* L.[2]
		Intermediate wheatgrass, *Thinopyrum intermedium* Host[4]
		Wheat, *Triticum aestivum* L.[3,4]
		Spelt, *Triticum spelta* L.[4]

[1]Oyediran et al. 2004. [2]Wilson and Hibbard 2004. [3]Clark and Hibbard 2004. [4]Branson and Ortman 1970. [5]Spencer and Raghu 2009.

CHAPTER 2: EXAMINATION OF POTENTIAL DIFFERENCES BETWEEN ROTATION-RESISTANT AND ROTATION-SUSCEPTIBLE POPULATIONS OF THE WESTERN CORN ROOTWORM IN THE FIELD

Introduction

Corn, *Zea mays* (L.), is an economically important crop grown throughout the world. In 2012, an estimated 170 million ha of corn was planted—the United States accounted for approximately 20% of this area (USDA FAS 2012). The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is a chrysomelid beetle that has become a widespread and significant pest of corn in the United States (Gray et al. 2009). Yield losses and control costs associated with the corn rootworm complex, which includes the western corn rootworm, the northern corn rootworm (*Diabrotica barberi* Smith & Lawrence), and the southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber), are estimated to exceed $1 billion in the United States each year (Metcalf 1986). However, this value is likely much larger today than when it was originally estimated due to a variety of factors—these include inflation, higher seed costs due to widespread use of genetically modified corn hybrids, and an increase in the market value of corn.

Western corn rootworm larvae injure corn plants by feeding on root tissue, which can reduce a plant's rate of photosynthesis (Godfrey et al. 1993; Riedell and Reese 1999; Urías-López et al. 2000). Root injury inhibits the utilization of soil moisture (Godfrey et al. 1993) and nitrogen (Spike and Tollefson 1989). Additionally, root injury can lead to colonization by phytopathogenic or saprophytic fungi (Bryson et al. 1953; Kurtz et al. 2010). Environmental factors (e.g., moisture stress or soil compaction) play a role in determining the severity of root injury (Spike and Tollefson 1988; Ellsbury et al. 1994). When root injury is severe, plants may become lodged (i.e., fail to stand upright)—root lodging may cause problems during harvest and can reduce yield by 11 to 34% (Spike and Tollefson 1991). Yield loss due to root injury or lodging is highly variable and influenced by a number of biotic and abiotic factors (Gray and Steffey 1998). Tinsley et al. (2013) estimated that for every node (i.e., circle of roots) consumed, a yield loss of approximately 15% can be expected.

Historically, oviposition by western corn rootworm females occurred predominantly in corn (Levine et al. 2002). As a result, rotating corn with a non-host crop (e.g., soybean, *Glycine max* [L.] Merr.) was a widely

successful management tactic. Crop rotation remained an effective western corn rootworm management tactic since it was first recommended in the early 1900s (Gillette 1912). While this tactic continues to be efficacious across much of the range of this pest, some populations have evolved resistance to crop rotation (Levine et al. 2002). Severe root injury to first-year corn was observed in Illinois (Piper City, Ford County) in 1987 (Levine and Oloumi-Sadeghi 1996). As late as 1989, root injury to first-year corn throughout most of Illinois remained at subeconomic levels (Steffey et al. 1992). By 1995, however, crop rotation failed as a management tactic in a number of counties in east-central Illinois and northwestern Indiana (Levine et al. 2002). Pierce and Gray (2006) suggested that rotation-resistance was facilitated by decades of crop rotation. According to Gray et al. (2009), the current range of the rotation-resistant population of this pest includes the northern two-thirds of Illinois and Indiana, as well as portions of eastern Missouri and Iowa; southern Wisconsin and Michigan; and western Ohio.

The management of several key insect pests of corn changed in 1996 with the commercial introduction of genetically modified hybrids, which express insecticidal proteins derived from the soil-borne bacterium *Bacillus thuringiensis* Berliner (Gill et al. 1992). *Bacillus thuringiensis* (Bt) naturally produces a variety of insecticidal crystal proteins, known as δ-endotoxins. These proteins are produced as protoxins, ingested by the insect, and solubilized in the midgut to produce an active toxin. Binding of this toxin to the tissue lining the insect's midgut results in perforations and subsequent death (Gill et al. 1992). While the first Bt toxins primarily targeted the European corn borer, *Ostrinia nubilalis* Hübner, the first Bt toxin targeting the western corn rootworm was commercialized in 2003 (Cry3Bb1, Event MON-ØØ863-5, Monsanto Co., St. Louis, MO) (Head and Ward 2009).

When western corn rootworm larvae feed on corn roots expressing a Bt toxin, mortality can be significant (Storer et al. 2006; Hibbard et al. 2010a). However, Bt toxins targeting this pest are not considered to be "high-dose" (Siegfried et al. 2005). As a result, some larval survival, beetle emergence, and root injury can be expected for Bt hybrids (Gray and Steffey 2005). Adoption of Bt hybrids by farmers has increased dramatically since their commercialization (USDA ERS 2012). For the most part, Bt hybrids provide acceptable control of this insect (Tinsley et al. 2010). However, different Bt toxins vary in their ability to prevent root injury. Additionally, the ability of a particular Bt toxin to prevent root injury can vary significantly between hybrids (Gray et al. 2007) or over time (Vaughn et al. 2005). Recently, Gassmann et al. (2011) reported the first confirmation of field-evolved resistance to

a Bt toxin by the western corn rootworm. Survival on corn expressing Cry3Bb1 was approximately 3-times greater for resistant larvae than susceptible larvae.

Gray et al. (2007) evaluated a number of Bt hybrids targeting the western corn rootworm—all hybrids expressed the same Bt toxin (Cry3Bb1), which was inserted using the same transgenic event (MON-ØØ863-5). During one year of their experiment (2006), two trial sites were evaluated (Monmouth and Urbana, IL). Urbana is located in a region of Illinois that has historically had high populations of the rotation-resistant western corn rootworm; however, at Monmouth, rotation-resistant populations of this pest were much lower. Although root injury for the non-Bt untreated check was similar at both locations, root injury for the Bt hybrids was approximately 105% greater at the Urbana site. As a result, the authors hypothesized that rotation-resistant and rotation-susceptible larvae may differ in their capacity to injure roots. However, the experimental design used by Gray et al. (2007) was not constructed in a manner that could test this hypothesis explicitly—Bt hybrids at Monmouth and Urbana may have been subjected to feeding by both rotation-resistant and rotation-susceptible larvae. The primary goal of the field experiment described in the remaining sections of this chapter was to determine if rotation-resistant and rotation-susceptible western corn rootworm larvae differ with respect to their ability to injure Bt corn roots and cause yield loss. A second goal was to determine if these two populations of the western corn rootworm exhibit differences in emergence or fitness when exposed to Bt corn.

Materials and Methods

Experimental Design

This three-year experiment was conducted from 2010 through 2012 as a multiple location, randomized complete block design with four replications (blocks). Main locations included the Agricultural Engineering Farm at Urbana, Illinois, and the Haskell Agricultural Laboratory at Concord, Nebraska. A variety of different cropping sequences were used at each of the main locations. In 2010, cropping sequences included corn-after-corn (C/C) and corn-after-soybean (C/S). For the remainder of this chapter, any specific combination of main location and cropping sequence will be referred to as a "site." Main locations and cropping sequences were selected to isolate different populations of the western corn rootworm with respect to their susceptibility to crop rotation. In theory,

the Illinois C/C site contained some mixture of the rotation-resistant and rotation-susceptible populations, the Illinois C/S site contained only the rotation-resistant population, the Nebraska C/C site contained only the rotation-susceptible population, and the Nebraska C/S site contained neither the rotation-resistant nor rotation-susceptible populations. Due to poor results in 2010, corn-after-trap crop (C/T) was added as a cropping sequence in 2011 and 2012. Trap crops (e.g., late-planted corn and pumpkins) are commonly used to attract adults and enhance oviposition for trials evaluating western corn rootworm management products.

Five corn hybrids were evaluated at each site, including a hybrid expressing Cry34/35Ab1 (Event DAS-59122-7, P1162XR, DuPont Pioneer, Johnston, IA), its near-isoline (P1162HR, DuPont Pioneer), a hybrid expressing Cry3Bb1 (Event MON-88Ø17-3, DKC61-19, Monsanto Co.), its near-isoline (DKC61-22, Monsanto Co.), and a hybrid expressing both Cry34/35Ab1 and Cry3Bb1 (Events DAS-59122-7 and MON-88Ø17-3, DKC61-21, Monsanto Co.)—DKC61-19 and DKC61-21 shared a common near-isoline (DKC61-22). All seed was treated with a neonicotinoid insecticidal seed treatment (either clothianidin or thiamethoxam) at 0.25 mg active ingredient/seed. To simplify analyses, hybrids were pooled to create three treatments: (1) an "isoline" treatment that included hybrids without western corn rootworm-active insecticidal toxins, (2) a "single toxin" treatment that included hybrids expressing either Cry34/35Ab1 or Cry3Bb1, and (3) a "pyramid" treatment that included the hybrid expressing both Cry34/35Ab1 and Cry3Bb1.

Agronomic Information

Sites received nitrogenous fertilizer (anhydrous ammonia) at a rate of 200 kg N/ha. Conventional tillage practices were used at all sites (e.g., use of a chisel or disc plow in the fall and field cultivator in the spring). Weed management was performed through pre- and post-emergence applications of glyphosate. Planting dates ranged from 19 April to 25 May—these varied due to environmental conditions at planting time. Although planting equipment varied slightly across years, a four-row planter with 0.8 m row units was always used. Plots were 3.0 m wide by 9.1 m long and seeded at 93,900 seeds/ha. Replications were separated by 1.5 m wide bare soil alleys to facilitate sampling. Climatic information for Urbana and Concord was obtained for the duration of this experiment, as well as for the preceding 15-year period (1995–2009) (Midwestern Regional Climate Center, Champaign, IL). This information included mean temperature, cumulative heat units, cumulative heat stress units, and total

precipitation for the months of April through August and is reported in Tables 2.1 and 2.2 for Urbana and Concord, respectively.

Data Collection

Injury to corn roots caused by western corn rootworm larvae was quantified using the 0 to 3 node-injury scale (Oleson et al. 2005). This scale is linear and based on the proportion of nodal roots that exhibit injury. A "pruned" root is one that has been pruned back to within 3.8 cm of the stalk (or soil line if the root originated aboveground). Node-injury evaluations were performed when corn plants reached the R1 growth stage (silking)—dates ranged from 5 to 22 July. Five randomly selected root systems were extracted from the fourth row of each plot, washed, and rated for injury. The percentage of root systems with a node-injury rating less than 0.25 was determined for each plot (i.e., consistency percentage). Yields were estimated by mechanically harvesting the center two rows of each plot once corn plants reached the R6 growth stage (physiological maturity) and grain moisture reached approximately 20%—dates ranged from 7 to 27 October. To ensure uniform populations in the harvested rows, plots were thinned to 79,100 plants/ha at the V6 growth stage (six leaf collar)—dates ranged from 14 to 28 June. Due to extreme drought conditions experienced at Urbana and Concord in 2012 (see Tables 2.1 and 2.2), plot yields were unable to be estimated. As a substitute, barren stalk percentages were determined by counting the number of corn plants without an ear for 5.3 m in the second row of each plot. Barren stalk assessments were performed at the R6 growth stage—28 September for all sites.

Prior to the initial emergence of western corn rootworm beetles, three single-plant emergence cages (described in detail by Pierce and Gray 2007) were deployed in the first row of each plot—dates ranged from 5 to 27 June. A photograph of this style of emergence cage can be viewed in Figure 2.1. Beetles were removed from the cages twice per week throughout the growing season until emergence concluded. There were typically 15 to 20 sampling dates/site. Immediately after removal from the cages, beetles were stored at –18 °C for processing at a later date. When emergence concluded for all sites, the sex of each beetle was determined. Female and male beetles can be differentiated by a sexual dimorphism on the posterior tip of the abdomen (White 1977). Males possess a supra-anal sclerite that causes their abdomen to appear blunt when viewed laterally; females lack this sclerite and their abdomen appears pointed in lateral view. Additionally, males have distinctively longer antennae

than females (Krysan 1986). After sex determination, cumulative female and male emergence for each plot was calculated by summing the numbers of each sex captured across all sampling dates. Additionally, a female-to-male sex ratio was calculated for each plot by dividing the cumulative number of females by the cumulative number of males. Sex ratios were not calculated for sites with exceedingly low beetle recovery (fewer than 100 beetles captured across all sampling dates).

To estimate fitness, head capsule widths and weights were recorded for all beetles recovered. Head capsule widths were determined for each beetle by measuring the widest portion of the head, laterally, from the left edge of left eye to the right edge of the right eye. A dissecting microscope (Model MZ125, Leica Microsystems Inc., Buffalo Grove, IL) with an ocular reticle was used—a magnification of 8X was selected to achieve an accuracy of 10 µm. After measuring head capsule widths, beetles were placed in an oven (Thelco Model 28, Precision Scientific Co., Chicago, IL) for 24 h at 60 °C. This step ensured that all beetles reached a uniform moisture concentration prior to weighing. Beetles were weighed using a balance (Model XS104, Mettler-Toledo Inc., Columbus, OH) providing an accuracy of 0.1 mg.

Statistical Analysis

Response variables (node-injury ratings, consistency percentages, yields, barren stalk percentages, cumulative emergence, sex ratios, head capsule widths, and weights) were analyzed using SAS 9.2 (SAS Institute Inc., Cary, NC). To stabilize variances and meet the assumptions of analysis of variance, all response variables were analyzed using a square-root transformation. Statistical tests for fixed effects were performed using PROC MIXED. Fixed effects were declared significant at $P \leq 0.05$. For all response variables excluding head capsule widths and weights, fixed effects included site, treatment, and their interaction. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D) (Kenward and Roger 1997).

Two challenges were encountered when analyzing head capsule widths and weights. First, the number of observations for these response variables at some sites were too few to obtain an estimate for error in the model. For example, 85% of plots across all sites in 2010 had a cumulative total emergence of no more than a single beetle. Without a greater number of beetles, mean head capsule widths or weights were nonestimable. A related, albeit distinct, second challenge stemmed from the fact that not all treatment combinations were present during

each year. At least some observations for every treatment combination are necessary to estimate the effect of the interaction between site and treatment. These challenges were addressed by analyzing head capsule widths and weights on a per site basis (i.e., each site was analyzed independently). As a result, the only fixed effect for these two response variables was treatment. Means for all response variables were compared using the LSMEANS option of PROC MIXED. Means were declared significantly different at $P \leq 0.05$.

Results

Node-Injury Ratings

Across all years of this experiment, the effects of site, treatment, and their interaction on mean node-injury ratings were significant (Table 2.3). For this and all subsequent response variables, the focus of the results will be on the interaction effect when significant rather than on the independent main effects of site and treatment. Conversely, results will focus on the independent main effects when the interaction of site and treatment was not significant. In 2010, mean node-injury ratings were minimal, ranged from 0.00 to 0.07, and were statistically similar across treatments with respect to the Illinois C/C, Illinois C/S, and Nebraska C/S sites (Table 2.4). At the Nebraska C/C site, the isoline treatment had a significantly greater mean node-injury rating than the single toxin and pyramid treatments, which had statistically similar mean node-injury ratings (Table 2.4).

In 2011, mean node-injury ratings were substantially greater for some sites, ranged from 0.00 to 1.46, and were statistically similar across treatments with respect to the Illinois C/S, Illinois C/T, Nebraska C/C, and Nebraska C/S sites (Table 2.5). At the Illinois C/C and Nebraska C/T sites, the isoline treatment had a significantly greater mean node-injury rating than the single toxin and pyramid treatments, which had statistically similar mean node-injury ratings (Table 2.5).

In 2012, mean node-injury ratings ranged from 0.00 to 2.04 and were statistically similar across treatments with respect to the Illinois C/S, Nebraska C/C, Nebraska C/S, and Nebraska C/T sites (Table 2.6). At the Illinois C/C and Illinois C/T sites, the isoline treatment had a significantly greater mean node-injury rating than the single toxin and pyramid treatments (Table 2.6). At the Illinois C/C site, the single toxin and pyramid treatments had

statistically similar mean node-injury ratings; however, the single toxin treatment had a significantly greater mean node-injury rating than the pyramid treatment at the Illinois C/T site (Table 2.6).

Consistency Percentages

In 2010, the effects of site, treatment, and their interaction on mean consistency percentages were not significant (Table 2.3). With respect to the effect of site, mean consistency percentages ranged from 98 to 100% across sites and were statistically similar. Regarding the effect of treatment, mean consistency percentages ranged from 99 to 100% across treatments and were statistically similar.

In 2011 and 2012, the effects of site, treatment, and their interaction on mean consistency percentages were significant (Table 2.3). In 2011, mean consistency percentages ranged from 20 to 100% and were statistically similar across treatments with respect to the Illinois C/S, Illinois C/T, Nebraska C/C, and Nebraska C/S sites (Table 2.5). At the Illinois C/C and Nebraska C/T sites, the isoline treatment had a significantly lower mean consistency percentage than the single toxin and pyramid treatments, which had statistically similar mean consistency percentages (Table 2.5).

In 2012, mean consistency percentages ranged from 3 to 100% and were statistically similar across treatments with respect to the Illinois C/S, Nebraska C/C, Nebraska C/S, and Nebraska C/T sites (Table 2.6). At the Illinois C/C and Illinois C/T sites, the isoline treatment had a significantly lower mean consistency percentage than the single toxin and pyramid treatments (Table 2.6). At the Illinois C/C site, the single toxin and pyramid treatments had statistically similar mean consistency percentages; however, the single toxin treatment had a significantly lower mean consistency percentage than the pyramid treatment at the Illinois C/T site (Table 2.6).

Yields

In 2010, the effects of site and treatment on mean yields were significant—the effect of their interaction was not (Table 2.3). With respect to the effect of site, the Illinois C/C site (7.9 t/ha) had a significantly lower mean yield than all other sites. The Nebraska C/C site (14.7 t/ha) had the greatest mean yield; however, this site and the Nebraska C/S site (13.8 t/ha) had statistically similar mean yields. The Illinois C/S site (12.5 t/ha) had a significantly lower mean yield than the Nebraska C/C site but not the Nebraska C/S site. Regarding the effect of treatment, the

single toxin treatment (11.9 t/ha) had a significantly greater mean yield than the isoline and pyramid treatments (each averaging 11.6 t/ha), which had statistically similar mean yields.

In 2011, the effects of site, treatment, and their interaction on mean yields were significant (Table 2.3). Mean yields ranged from 7.7 to 13.7 t/ha and were statistically similar across treatments with respect to the Illinois C/S, Illinois C/T, Nebraska C/C, and Nebraska C/S sites (Table 2.5). At the Illinois C/C site, the isoline treatment had a significantly lower mean yield than the single toxin and pyramid treatments, which had statistically similar mean yields (Table 2.5). At the Nebraska C/T site, the single toxin treatment had a significantly greater mean yield than the isoline and pyramid treatments, which had statistically similar mean yields (Table 2.5).

Barren Stalk Percentages

In 2012, the effects of site, treatment, and their interaction on mean barren stalk percentages were significant (Table 2.3). Mean barren stalk percentages ranged from 0 to 100% and were statistically similar across treatments with respect to the Illinois C/S, Nebraska C/C, Nebraska C/S, and Nebraska C/T sites (Table 2.6). At the Illinois C/C and Illinois C/T sites, the isoline treatment had a significantly greater mean barren stalk percentage than the single toxin and pyramid treatments (Table 2.6). At the Illinois C/C site, the single toxin and pyramid treatments had statistically similar mean barren stalk percentages; however, the single toxin treatment had a significantly greater mean barren stalk percentage than the pyramid treatment at the Illinois C/T site (Table 2.6).

Cumulative Female Emergence

In 2010, the effect of site on mean cumulative female emergence was significant—the effects of treatment and their interaction were not (Table 2.7). Mean numbers of females/plot were exceptionally low in 2010. With respect to the effect of site, mean numbers of females/plot never exceeded a single beetle. Statistically, the Illinois C/C and Illinois C/S sites (each averaging 1 female/plot) had significantly greater mean numbers of females/plot than the Nebraska C/C and Nebraska C/S sites (each averaging 0 females/plot). Regarding the effect of treatment, mean numbers of females/plot ranged from 0 to 1 and were statistically similar across treatments.

In 2011, the effects of site, treatment, and their interaction on mean cumulative female emergence were significant (Table 2.7). Mean numbers of females/plot ranged from 0 to 28 and were statistically similar across treatments with respect to the Illinois C/S, Nebraska C/C, Nebraska C/S, and Nebraska C/T sites (Table 2.8). At the

Illinois C/C and Illinois C/T sites, the isoline treatment had a significantly greater mean number of females/plot than the single toxin and pyramid treatments, which had statistically similar mean numbers of females/plot (Table 2.8).

In 2012, the effects of site and treatment on mean cumulative female emergence were significant—the effect of their interaction was not (Table 2.7). With respect to the effect of site, the Nebraska C/S site (0 females/plot) had a significantly lower mean number of females/plot than all other sites. The Illinois C/T site (30 females/plot) had a significantly greater mean number of females/plot than all other sites, excluding the Illinois C/C site (20 females/plot). This latter site had a significantly greater mean number of females/plot than the Nebraska C/C site (9 females/plot), but not the Illinois C/S and Nebraska C/T sites (17 and 16 females/plot, respectively). The Nebraska C/T and Nebraska C/C sites had statistically similar mean numbers of females/plot. Regarding the effect of treatment, the pyramid treatment (6 females/plot) had a significantly lower mean number of females/plot than the isoline and single toxin treatments (20 and 15 females/plot, respectively), which had statistically similar mean numbers of females/plot.

Cumulative Male Emergence

In 2010, the effects of site, treatment, and their interaction on mean cumulative male emergence were not significant (Table 2.7). As was the case for cumulative female emergence, mean numbers of males/plot were exceedingly low in 2010. In fact, mean numbers of males/plot never exceeded a single beetle for the effects of site and treatment—no significant differences were observed.

In 2011, the effects of site, treatment, and their interaction on mean cumulative male emergence were significant (Table 2.7). Mean numbers of males/plot ranged from 0 to 15 and were statistically similar across treatments with respect to the Illinois C/S, Nebraska C/C, and Nebraska C/S sites (Table 2.8). At the Illinois C/C, Illinois C/T, and Nebraska C/T sites, the isoline treatment had a significantly greater mean number of males/plot than the single toxin and pyramid treatments (Table 2.8). At the Illinois C/T and Nebraska C/T sites, the single toxin and pyramid treatments had statistically similar mean numbers of males/plot; however, the single toxin treatment had a significantly greater mean number of males/plot than the pyramid treatment at the Illinois C/C site (Table 2.8).

In 2012, the effects of site and treatment on mean cumulative male emergence were significant—the effect of their interaction was not (Table 2.7). With respect to the effect of site, the Illinois C/T site (21 males/plot) had a significantly greater mean number of males/plot than all other sites. The Illinois C/C site (13 males/plot) had a significantly greater mean number males/plot than all remaining sites. The Illinois C/S, Nebraska C/C, and Nebraska C/T sites (7, 4, and 7 males/plot, respectively) had statistically similar mean numbers of males/plot, which were significantly greater than the mean number of males/plot at the Nebraska C/S site (0 males/plot). Regarding the effect of treatment, the isoline treatment (13 males/plot) had a significantly greater mean number of males/plot than the single toxin or pyramid treatments (3 and 7 males/plot). Mean numbers of males/plot differed significantly for these latter two treatments.

Female-to-Male Sex Ratios

In 2010, an insufficient number of beetles were recovered to calculate mean sex ratios. However, mean sex ratios were calculated for 50 and 83% of all sites in 2011 and 2012, respectively. In 2011, the effects of site, treatment, and their interaction on mean sex ratios were not significant (Table 2.9). With respect to the effect of site, mean sex ratios were only calculated for the Illinois C/C, Illinois C/T, and Nebraska C/T sites (2.47, 2.07, and 3.13 females/male, respectively)—no significant differences were observed. Regarding the effect of treatment, mean sex ratios ranged from 2.34 to 2.70 females/male and were statistically similar across treatments.

In 2012, the effects of site and treatment on mean sex ratios were significant—the effect of their interaction was not (Table 2.9). With respect to the effect of site, the Illinois C/T site (1.72 females/male) had a significantly lower mean sex ratio than all other sites. The mean sex ratio for the Illinois C/C site (2.18 females/male) was significantly lower than the mean sex ratio for the Nebraska C/C site (3.81 females/male). Mean sex ratios for the Illinois C/S and Nebraska C/T sites (each averaging 3.35 females/male) were statistically similar to the mean sex ratios for the Illinois C/C and Nebraska C/C sites. A mean sex ratio could not be calculated for the Nebraska C/S site. Regarding the effect of treatment, the isoline treatment (1.98 females/male) had a significantly lower mean sex ratio than the single toxin and pyramid treatments (3.26 and 3.84 females/male, respectively), which had statistically similar mean sex ratios.

Female Head Capsule Widths

In 2010, the Illinois C/C site experienced a significant effect of treatment on mean head capsule widths for females (Table 2.10). At this site, females from the pyramid treatment had a significantly greater mean head capsule width than females from the isoline and single toxin treatments, which had females with statistically similar mean head capsule widths (Table 2.11). At the Illinois C/S site, mean head capsule widths for females ranged from 1190 to 1220 µm and were statistically similar across treatments (Table 2.11). No females were recovered at the Nebraska sites. In 2011, none of the sites experienced a significant effect of treatment on mean head capsule widths for females (Table 2.10). Across all sites, mean head capsule widths for females ranged from 1040 to 1170 µm and were statistically similar across treatments with respect to site (Table 2.12).

In 2012, the Illinois sites experienced a significant effect of treatment on mean head capsule widths for females (Table 2.10). At the Illinois C/C and Illinois C/S sites, females from the isoline treatment had a significantly greater mean head capsule width than females from the single toxin and pyramid treatments, which had females with statistically similar mean head capsule widths (Table 2.13). At the Illinois C/T site, females from the isoline and pyramid treatments had significantly different mean head capsule widths; however, the mean head capsule width for females from the single toxin treatment did not differ significantly from the mean head capsule widths for females from the isoline and pyramid treatments (Table 2.13). Across the Nebraska sites, mean head capsule widths for females ranged from 1120 to 1190 µm and were statistically similar across treatments with respect to site (Table 2.13).

Male Head Capsule Widths

In 2010, an insufficient number of males were recovered to calculate mean head capsule widths. In 2011, none of the sites experienced a significant effect of treatment on mean head capsule widths for males (Table 2.14). Across all sites, mean head capsule widths for males ranged from 1030 to 1150 µm and were statistically similar across treatments with respect to site (Table 2.12).

In 2012, the Illinois C/C, Illinois C/S, and Nebraska C/C sites experienced a significant effect of treatment on mean head capsule widths for males (Table 2.14). At these sites, males from the pyramid treatment had a significantly smaller mean head capsule width than males from the isoline and single toxin treatments (Table 2.13).

At the Illinois C/C site, males from the isoline treatment had a significantly greater mean head capsule width than males from the single toxin treatment; however, at the Illinois C/S and Nebraska C/C sites, males from the isoline and single toxin treatments had statistically similar mean head capsule widths (Table 2.13). Across the remaining sites, mean head capsule widths for males ranged from 1100 to 1160 µm and were statistically similar across treatments with respect to site (Table 2.13).

Female Weights

In 2010 and 2011, none of the sites experienced a significant effect of treatment on mean weights for females (Table 2.15). Across all sites in 2010, mean weights for females ranged from 1.0 to 4.0 mg and were statistically similar across treatments with respect to site (Table 2.11). Similarly, across all sites in 2011, mean weights for females ranged from 1.9 to 3.7 mg and were statistically similar across treatments with respect to site (Table 2.12).

In 2012, the Illinois C/S site experienced a significant effect of treatment on mean weights for females (Table 2.15). At this site, females from the pyramid treatment had a significantly greater mean weight than females from the single toxin treatment (Table 2.13). The mean weight for females from the isoline treatment was statistically similar to the mean weights for females from the single toxin and pyramid treatments (Table 2.13). Across the remaining sites, mean weights for females ranged from 1.8 to 3.8 mg and were statistically similar across treatments with respect to site (Table 2.13).

Male Weights

In 2010, an insufficient number of males were recovered to calculate mean weights. In 2011, the Illinois C/T and Nebraska C/C sites experienced a significant effect of treatment on mean weights for males (Table 2.16). At these sites, males from the isoline treatment had a significantly greater mean weight than males from the single toxin treatment (Table 2.12). At the Illinois C/T site, the mean weight for males from the pyramid treatment did not differ significantly from the mean weights for males from the isoline and single toxin treatments (Table 2.12). At the Nebraska C/C site, an insufficient number of males were recovered from the pyramid treatment to calculate a mean weight. Across the remaining sites, mean weights for males ranged from 1.4 to 2.1 mg and were statistically similar across treatments with respect to site (Table 2.12).

In 2012, the Illinois C/S, Illinois C/T, and Nebraska C/T sites experienced a significant effect of treatment on mean weights for males (Table 2.16). At these sites, the mean weight for males from the isoline treatment was significantly greater than the mean weight for males from the pyramid treatment and statistically similar to the mean weight for males from the single toxin treatment (Table 2.13). At the Illinois C/S site, males from the single toxin treatment had a significantly greater mean weight than males from the pyramid treatment; however, at the Illinois C/T and Nebraska C/T sites, males from the single toxin and pyramid treatments had statistically similar mean weights (Table 2.13). Across the remaining sites, mean weights for males ranged from 2.2 to 3.2 mg and were statistically similar across treatments with respect to site (Table 2.13).

Discussion

Mean node-injury ratings were exceptionally low in 2010. Across locations, mean node-injury ratings for all treatments, including the isoline treatment, were below 0.10 (Table 2.4). In 2010, both Urbana and Concord experienced an excessive amount of rainfall during June—10.6 cm more than normal (Tables 2.1 and 2.2). Because western corn rootworm larvae typically hatch from late May to early June (Bryson et al. 1953), the excessive rainfall likely resulted in saturated soil conditions coinciding with larval hatch. Such conditions reduce successful larval establishment on corn roots and increase mortality (Sutter et al. 1989). The relative absence of root injury resulted in very high mean consistency percentages and yields. Although significant differences in mean yields were observed for the effects of site and treatment, it is unlikely that these differences were related to root injury. For example, two replications at the Illinois C/C site experienced standing water throughout much of the early part of the growing season, which resulted in a substantially lower mean yield when compared with other sites. It is uncertain why significant differences in mean yields were observed for the effect of treatment—mean node-injury ratings were mostly similar across treatments (Table 2.4). Although differences were observed, the range in mean yields was relatively small for the effect of treatment (11.6 to 11.9 t/ha).

Cumulative emergence of western corn rootworm beetles was poor during 2010. In fact, no beetles were recovered at the Nebraska sites. At Illinois, no site or treatment averaged more than a single beetle/plot. Because so few beetles were recovered, female-to-male sex ratios could not be calculated. Additionally, too few males

were recovered to calculate mean head capsule widths and weights. Although these fitness measurements were calculated for females, sample sizes for the various treatments were quite low. Some significant differences were observed in mean head capsule widths and weights for females (Table 2.11), although trends were inconsistent. For example, females from the pyramid treatment at the Illinois C/C site had a significantly greater mean head capsule width than females from the isoline and single toxin treatments (Table 2.11). However, this difference in mean head capsule widths did not translate into a difference in mean weights (Table 2.11). Overall, the interpretation of fitness measurements in 2010 was negatively affected by the extremely low number of beetles recovered.

As in 2010, mean node-injury ratings were exceptionally low at many sites in 2011 (Table 2.5). For these sites, most notably the Illinois C/S, Illinois C/T, and Nebraska C/C sites, it is unclear why root injury was minimal. Near-optimal weather conditions were observed during the time period when western corn rootworm larvae were establishing on corn roots at both Urbana and Concord (Tables 2.1 and 2.2). One potential explanation for the small amount of root injury observed at these sites in 2011 could be a lack of abundant oviposition by females during the previous growing season. Beetle movement is strongly influenced by corn phenology (Darnell et al. 2000; Campbell and Meinke 2006). If these sites were located near a more attractive feeding location in 2010 (e.g., a pollinating cornfield), females may have been drawn away from the trial sites prior to laying their eggs. However, two sites did experience a substantial amount of root injury—the Illinois C/C and Nebraska C/T sites. At these sites, mean node-injury ratings for the isoline treatment were 0.94 and 1.46, respectively (Table 2.5). Notably, mean node-injury ratings for the single toxin and pyramid treatments were statistically similar at these sites (Table 2.5).

Mean consistency percentages in 2011 could generally be explained by examining mean node-injury ratings—treatments with substantial root injury experienced reduced consistency, and vice versa (Table 2.5). To a lesser extent, mean node-injury ratings could be used to explain mean yields. Treatments with more root injury tended to have lower yields (Table 2.5). This trend excluded the pyramid treatment at the Nebraska C/T site (Table 2.5). It is uncertain why mean yields for the pyramid and isoline treatments were statistically similar, considering the substantial difference in mean node-injury ratings for these two treatments (1.27 nodes, Table 2.5). In general, the Nebraska sites had greater mean yields than the Illinois sites (Table 2.5)—some difference in the agronomic potential of these two main locations was anticipated due to their large geographical separation (nearly 800 km).

However, this trend excluded the Nebraska C/T site. During 2011, corn plants at this site experienced significant competition with weeds that were difficult to control.

Most of the western corn rootworm beetles recovered in 2011 were from the Illinois C/C site. Mean cumulative emergence for the remaining sites never exceeded 10 beetles/plot. Of particular interest was the observation that the isoline treatment at the Illinois C/C site had significantly greater mean numbers of beetles/plot than the isoline treatment at the Nebraska C/T site (Table 2.8). These sites were the only two at which a substantial amount of root injury was observed for the isoline treatment (Table 2.5). However, the isoline treatment at the Illinois C/C site had a significantly lower mean node-injury rating than the isoline treatment at the Nebraska C/T site (Table 2.5). Because the difference in mean node-injury ratings for the isoline treatment at these two sites was only 0.52 (Table 2.5), it is unlikely that larvae at the Nebraska C/T site experienced substantially more density-dependent mortality than larvae at the Illinois C/C site. Although density-dependent mortality can negatively affect beetle emergence (Branson and Sutter 1985; Onstad et al. 2006), Hibbard et al. (2010b) noted that egg densities need to be very high (2500 viable eggs/m of row) to observe density-dependent effects.

Female-to-male sex ratios were only calculated for 50% of the sites in 2011 and were skewed toward a greater number of females than males for both the effects of site and treatment—no significant differences were observed. The lack of statistical separation among sites and treatments may indicate that the sex ratio for western corn rootworm beetles is not a measurement that is highly sensitive to change during situations in which few beetles are recovered. This hypothesis agrees with the findings of Weiss et al. (1985), who observed a significant difference in the sex ratio for this pest when comparing beetle emergence from lightly and heavily injured roots. Across all sites in 2011, mean head capsule widths for females and males, as well as mean weights for females, were statistically similar across treatments (Table 2.12). However, some significant differences among treatments in mean weights for males were observed at the Illinois C/T and Nebraska C/C sites (Table 2.12). At these sites, the mean weight for males from the single toxin treatment was significantly lower than for males from the isoline treatment (Table 2.12). However, the numbers of males/plot recovered at these sites were extremely low (never averaging more than 8 for any treatment, Table 2.8). For a situation in which more beetles were recovered (e.g., at the Illinois C/C site), significant differences were not observed in mean weights for males (Table 2.12).

Not unlike 2010 and 2011, mean node-injury ratings were exceedingly low at most sites in 2012 (Table 2.6). In fact, a substantial amount of root injury for the isoline treatment was only observed at the Illinois C/T site (Table 2.6). As was the case in 2011, the relative absence of root injury at some sites, in particular the Illinois C/C, Illinois C/S, Nebraska C/C, and Nebraska C/T sites, is difficult to explain. No evidence for substantial mortality caused by environmental factors during western corn rootworm larval establishment on corn roots is present when examining climactic conditions in 2012 (Tables 2.1 and 2.2). As in 2011, a possible explanation for the small amount of root injury observed at these sites in 2012 could be a lack of abundant oviposition by females during the previous growing season.

Mean consistency percentages were highly correlated with root injury—treatments with smaller mean node-injury ratings had much higher mean consistency percentages (Table 2.6). Conversely, mean barren stalk percentages were not closely associated with root injury (Table 2.6). Although it may be intuitive to hypothesize that treatments with substantial stress (e.g., root injury) would experience an increased mean barren stalk percentage, this was not observed (Table 2.6). For example, although mean node-injury ratings for the isoline treatment at the Illinois C/C and Illinois C/T sites differed significantly, their mean barren stalk percentages were statistically similar (Table 2.6). Additionally, virtually no root injury was observed at the Nebraska C/C and Nebraska C/T sites, yet mean barren stalk percentages at these sites were substantial (Table 2.6). If associated, the relationship between root injury and the ability of a corn plant to produce an ear is weak.

Similar to previous years, cumulative emergence of western corn rootworm beetles was low in 2012. Mean numbers of females and males per plot never exceeded 30 and 15, respectively. As in 2011, an interesting trend was observed with regard to root injury and cumulative emergence, at least for females. Two sites that experienced dramatically different amounts of root injury had similar female cumulative emergence (the Illinois C/C and Illinois C/T sites). Observing this trend for two years of data may suggest that the relationship between root injury and beetle emergence deserves further investigation. Perhaps the relationship between these two response variables is more complicated than one would assume—at least for situations in which few beetles are recovered. Across sites, significantly more males/plot were recovered from the isoline treatment than from the single toxin and pyramid treatments. However, this trend was not observed for females. This observation may suggest that cumulative female and male emergence is differentially affected by a single toxin Bt hybrid.

In 2012, the site with the most root injury (the Illinois C/T site) had the lowest female-to-male sex ratio. Additionally, when averaged across sites, the isoline treatment had a lower sex ratio than the single toxin and pyramid treatments. Although these observations may suggest that the sex ratio is skewed toward fewer females per male for situations in which root injury is substantial, this trend was not observed in 2011. Without a clear and consistent trend across years, it is difficult to determine how the sites and treatments evaluated during this experiment affected the ratio between female and male beetles.

Overall trends in fitness measurements for western corn rootworm beetles in 2012 were difficult to detect. For example, sites with similar levels of root injury and cumulative emergence (e.g., the Illinois C/S, Nebraska C/C, and Nebraska C/T sites) did not experience consistently similar responses in mean head capsule widths and weights for females and males (Table 2.13). At the site with the most root injury and cumulative emergence (the Illinois C/T site), conflicting fitness measurements were observed (Table 2.13). Females from the isoline treatment had a significantly greater mean head capsule width than females from the pyramid treatment, although their mean weights were statistically similar (Table 2.13). Conversely, males from the isoline treatment had a significantly greater mean weight than males from the pyramid treatment, but males from these treatments had statistically similar mean head capsule widths (Table 2.13). Again, without clear and consistent trends across years, it is difficult to determine how fitness measurements were affected by the sites and treatments evaluated during this experiment.

The primary goal of this experiment was to determine if rotation-resistant and rotation-susceptible western corn rootworm larvae differ with respect to their ability to injure Bt corn roots and cause yield loss. A second goal was to determine if these two populations of the western corn rootworm exhibit differences in emergence or fitness when exposed to Bt corn. Unfortunately, these goals were difficult to achieve—the success of this experiment (with regard to achieving these two goals) was fundamentally dependent on field data that was collected. Substantial and similar levels of root injury for the isoline treatment across sites (excluding the Nebraska C/S site) were required. However, this pattern of root injury was never observed—mean node-injury ratings were minimal across sites and treatments in 2010 (Table 2.4) and substantial root injury for the isoline treatment was only observed at two sites in 2011 (Table 2.5) and at one site in 2012 (Table 2.6).

Without first observing substantial and similar levels of root injury for the isoline treatment across sites, the ability to draw conclusions regarding potential differences between rotation-resistant and rotation-susceptible populations of the western corn rootworm was reduced. Confounding factors (e.g., density-dependent mortality or environmental variation) inhibited making meaningful comparisons when significant differences in root injury for the isoline treatment were observed across sites. Although this experiment was designed in such a way to make the desired comparisons, field data collected did not allow for substantive conclusions to be drawn. This highlights the limited usefulness of performing a field experiment to answer questions that may require examination on a finer scale. In the third chapter of this dissertation, a greenhouse experiment is described that attempts to answer similar questions regarding differences between rotation-resistant and rotation susceptible populations of the western corn rootworm. This greenhouse experiment was able to control for potentially confounding factors.

The substantial variability observed for cumulative emergence, female-to-male sex ratios, and fitness measurements, accompanied by the relative difficulty associated with identifying meaningful, consistent trends, may have implications for the utility of these response variables. Results from this experiment may indicate that evaluating these response variables is of limited value when root injury and beetle emergence is minimal. Additionally, the style of emergence cage used during this experiment has been used successfully in previous experiments examining western corn rootworm emergence (e.g., Pierce and Gray 2007); however, to my knowledge, the utility of single-plant emergence cages has not been thoroughly evaluated for situations in which beetle emergence is exceedingly low. Perhaps another beetle recovery technique may be more appropriate for such a situation—an example of which may be a large, walk-in screen house that can be placed over many rows of corn plants (e.g., Hibbard et al. 2010b). Because the spatial distribution of western corn rootworm eggs in a field can be highly aggregated (Park and Tollefson 2006), large screen houses may provide a more representative sample than single-plant emergence cages when evaluating beetle emergence, and subsequently sex ratios and fitness measurements, when emergence is minimal.

Throughout this experiment, there were many sites where root injury was unexpectedly low. In 2010, minimal root injury was easily explained by examining climatic information during western corn rootworm larval establishment. However, in subsequent years, the relative lack of root injury (most notably for the isoline treatment) at some sites was difficult to explain. The virtual absence of root injury at many sites where corn did

not follow trap crop gives rise to the following question: are current management practices targeting the western corn rootworm being used justifiably? Gray (2012) concisely outlined the current state of management regarding this key pest:

> "In 2003, Bt hybrids were commercialized that offered protection against corn rootworm larval injury. These hybrids became the dominant management approach for corn rootworms on both rotated and nonrotated corn acres. Fueled by growing concerns over lackluster Bt hybrid performance in some areas of the Corn Belt, it is becoming more common for growers to use Bt corn rootworm hybrids and to treat those hybrids at planting with a soil insecticide. Many producers also are inclined to broadcast insecticide applications on their fields tank-mixed with fungicides during pollination. The argument for these treatments includes protecting silks against clipping by western corn rootworm adults and Japanese beetles. Many producers freely admit, though, that they also want to 'take the edge off' of the egg-laying population of western corn rootworm adults to minimize the pressure placed on soil insecticides and Bt hybrids the following growing season."

There is a substantial disconnect between the need for and use of aggressive management for this important pest in recent years. Gray (2011) reported on the results of a recent statewide survey for western corn rootworm beetles in cornfields throughout Illinois. Results from this survey (conducted in late July and early August, 2011) demonstrated that densities of beetles were exceptionally low. In fact, densities of beetles never exceeded a single beetle/plant for any of the counties sampled. Densities of more than 0.5 beetles/plant were only observed for 4% of the counties surveyed. Such low densities of western corn rootworm beetles suggest that there may be an economic benefit available to farmers who choose to scout for this key pest during the growing season and make their management decisions based upon established thresholds. If the western corn rootworm continues to be managed aggressively, serious, negative consequences for the durability of current management practices will likely result (Gassmann 2012), as have been documented previously for crop rotation (Levine et al. 2002), various classes of insecticides (Hamilton 1965; Ball 1968; Meinke et al. 1998), and, most recently, a Bt toxin (Cry3Bb1, Gassmann et al. 2011).

References

Ball HJ, 1968. A five-year study of potential western corn rootworm resistance to diazinon and phorate in Nebraska. J. Econ. Entomol. 61, 496–498.

Branson TF, Sutter GR, 1985. Influence of population density of immatures on size, longevity, and fecundity of adult *D. virgifera virgifera* (Coleoptera: Chrysomelidae). Environ. Entomol. 14, 687–690.

Bryson HR, Wilbur DA, Burkhardt CC, 1953. The western corn rootworm, *Diabrotica virgifera* Lec. in Kansas. J. Econ. Entomol. 46, 995–999.

Campbell LA, Meinke LJ, 2006. Seasonality and adult habitat use by four *Diabrotica* species at prairie-corn interfaces. Environ. Entomol. 35, 922–936.

Darnell SJ, Meinke LJ, Young LJ, 2000. Influence of corn phenology on adult western corn rootworm (Coleoptera: Chrysomelidae) distribution. Environ. Entomol. 29, 587–595.

Ellsbury MM, Schumacher TE, Gustin RD, Woodson WD, 1994. Soil compaction effect on corn rootworm populations in maize artificially infested with eggs of western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 23, 943–948.

Gassmann AJ, 2012. Field-evolved resistance to Bt maize by western corn rootworm: predictions from the laboratory and effects in the field. J. Invertebr. Pathol. 110, 287–293.

Gassmann AJ, Petzold-Maxwell JL, Keweshan RS, Dunbar MW, 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLOS ONE 6, E22629.

Gill SS, Cowles EA, Pietrantonio PV, 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Annu. Rev. Entomol. 37, 615–636.

Gillette CP, 1912. *Diabrotica virgifera* Lec. as a corn root-worm. J. Econ. Entomol. 5, 364–366.

Godfrey LD, Meinke LJ, Wright RJ, 1993. Effects of larval injury by western corn rootworm (Coleoptera: Chrysomelidae) on gas exchange parameters of field corn. J. Econ. Entomol. 86, 1546–1556.

Gray ME, 2011. Survey of Illinois cornfields reveals exceedingly low densities of western corn rootworm adults. In: The Bulletin: Pest Management and Crop Development Information for Illinois, Issue No. 18. University of Illinois Extension, Urbana, IL, pp. 106–107.

Gray ME, 2012. New insights on the western corn rootworm's adaptation to crop rotation. In: The Bulletin: Pest Management and Crop Development Information for Illinois, Issue No. 17. University of Illinois Extension, Urbana, IL, pp. 107–108.

Gray ME, Sappington TW, Miller NJ, Moeser J, Bohn MO, 2009. Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54, 303–321.

Gray ME, Steffey KL, 1998. Corn rootworm (Coleoptera: Chrysomelidae) larval injury and root compensation of 12 maize hybrids: an assessment of the economic injury index. J. Econ. Entomol. 91, 723–740.

Gray ME, Steffey KL, 2005. Transgenic YieldGard rootworm hybrid stumbles in Urbana experiment: why? In: Proceedings of the 2005 Illinois Crop Protection Technology Conference. University of Illinois Extension, Urbana, IL, pp. 41–46.

Gray ME, Steffey KL, Estes RE, Schroeder JB, 2007. Responses of transgenic maize hybrids to variant western corn rootworm larval injury. J. Appl. Entomol. 131, 386–390.

Hamilton EW, 1965. Aldrin resistance in corn rootworm beetles. J. Econ. Entomol. 58, 297–300.

Head G, Ward D, 2009. Insect resistance in corn through biotechnology. In: Biotechnology in Agriculture and Forestry Volume 63: Molecular Genetic Approaches to Maize Improvement, ed. Kriz AL, Larkins BA. Springer-Verlag, Berlin, Germany, pp. 31–40.

Hibbard BE, Clark TL, Ellersieck MR, Meihls LN, El Khishen AA, Kaster V, Steiner H, Kurtz R, 2010a. Mortality of western corn rootworm larvae on MIR604 transgenic maize roots: field survivorship has no significant impact on survivorship of F1 progeny on MIR604. J. Econ. Entomol. 103, 2187–2196.

Hibbard BE, Meihls LN, Ellersieck MR, Onstad DW, 2010b. Density-dependent and density-independent mortality of the western corn rootworm: impact on dose calculations of rootworm-resistant Bt corn. J. Econ. Entomol. 103, 77–84.

Kenward MG, Roger JH, 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53, 983–997.

Krysan JL, 1986. Introduction: biology, distribution, and identification of pest *Diabrotica*. In: Methods for the Study of Pest *Diabrotica*, ed. Krysan JL, Miller TA. Springer, New York, NY, pp. 1–23.

Kurtz B, Karlovsky P, Vidal S, 2010. Interaction between western corn rootworm (Coleoptera: Chrysomelidae) larvae and root-infecting *Fusarium verticillioides*. Environ. Entomol. 39, 1532–1538.

Levine E, Oloumi-Sadeghi H, 1996. Western corn rootworm (Coleoptera: Chrysomelidae) larval injury to corn grown for seed production following soybeans grown for seed production. J. Econ. Entomol. 89, 1010–1016.

Levine E, Spencer JL, Isard SA, Onstad DW, Gray ME, 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Am. Entomol. 48, 94–107.

Meinke LJ, Siegfried BD, Wright RJ, Chandler LD, 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J. Econ. Entomol. 91, 594–600.

Metcalf RL, 1986. Foreword. In: Methods for the Study of Pest *Diabrotica*, ed. Krysan JL, Miller TA. Springer, New York, NY, pp. vii–xv.

Oleson JD, Park Y, Nowatzki TM, Tollefson JJ, 2005. Node-injury scale to evaluate root injury by corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98, 1–8.

Onstad DW, Hibbard BE, Clark TL, Crowder DW, Carter KG, 2006. Analysis of density-dependent survival of *Diabrotica* (Coleoptera: Chrysomelidae) in cornfields. Environ. Entomol. 35, 1272–1278.

Park YL, Tollefson JJ, 2006. Spatial distributions of corn rootworm (Coleoptera: Chrysomelidae) eggs and larvae: implications for sampling. J. Kansas Entomol. Soc. 79, 129–135.

Pierce CMF, Gray ME, 2006. Western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), oviposition: a variant's response to maize phenology. Environ. Entomol. 35, 423–434.

Pierce CMF, Gray ME, 2007. Population dynamics of a western corn rootworm (Coleoptera: Chrysomelidae) variant in east-central Illinois commercial maize and soybean fields. J. Econ. Entomol. 100, 1104–1115.

Riedell WE, Reese RN, 1999. Maize morphology and shoot CO_2 assimilation after root damage by western corn rootworm larvae. Crop Sci. 39, 1332–1340.

Siegfried BD, Vaughn TT, Spencer T, 2005. Baseline susceptibility of western corn rootworm (Coleoptera: Chrysomelidae) to Cry3Bb1 *Bacillus thuringiensis* toxin. J. Econ. Entomol. 98, 1320–1324.

Spike BP, Tollefson JJ, 1988. Western corn rootworm (Coleoptera: Chrysomelidae) larval survival and damage potential to corn subjected to nitrogen and plant density treatments. J. Econ. Entomol. 81, 1450–1455.

Spike BP, Tollefson JJ, 1989. Relationship of plant phenology to corn yield loss resulting from western corn rootworm (Coleoptera: Chrysomelidae) larval injury, nitrogen deficiency, and high plant density. J. Econ. Entomol. 82, 226–231.

Spike BP, Tollefson JJ, 1991. Yield response of corn subjected to western corn rootworm (Coleoptera: Chrysomelidae) infestation and lodging. J. Econ. Entomol. 84, 1585–1590.

Steffey KL, Gray ME, Kuhlman DE, 1992. Extent of corn rootworm (Coleoptera: Chrysomelidae) larval damage in corn after soybeans: search for the expression of the prolonged diapause trait in Illinois. J. Econ. Entomol. 85, 268–275.

Storer NP, Babcock JM, Edwards JM, 2006. Field measures of western corn rootworm (Coleoptera: Chrysomelidae) mortality caused by Cry34/35Ab1 proteins expressed in maize event 59122 and implications for trait durability. J. Econ. Entomol. 99, 1381–1387.

Sutter GR, Branson TF, Fisher JR, Elliott NC, Jackson JJ, 1989. Effect of insecticide treatments on root damage ratings of maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 82, 1792–1798.

Tinsley NA, Estes RE, Gray ME, 2010. Evaluation of products to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2010. In: On Target: 2010 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 5–11.

Tinsley NA, Estes RE, Gray ME, 2013. Validation of a nested error component model to estimate damage caused by corn rootworm larvae. J. Appl. Entomol. 137, 161–169.

Urías-López MA, Meinke LJ, Higley LG, Haile FJ, 2000. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on photosynthetic rate and vegetative growth of different types of maize. Environ. Entomol. 29, 861–867.

USDA ERS [United States Department of Agriculture Economic Research Service], 2012. Adoption of Genetically Engineered Crops in the US. United States Department of Agriculture, Washington DC.

USDA FAS [United States Department of Agriculture Foreign Agricultural Service], 2012. World Agricultural Production (12-12). United States Department of Agriculture, Washington DC.

Vaughn T, Cavato T, Brar G, Coombe T, DeGooyer T, Ford S, Groth M, Howe A, Johnson S, Kolacz K, Pilcher C, Purcell J, Romano C, English L, Pershing J, 2005. A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. Crop Sci. 45, 931–938.

Weiss MJ, Seevers KP, Mayo ZB, 1985. Influence of western corn rootworm (*D. virgifera virgifera*) larval densities and damage on corn rootworm survival, developmental time, size and sex ratio (Coleoptera: Chrysomelidae). J. Kansas Entomol. Soc. 58, 397–402.

White R, 1977. Sexual characters of species of *Diabrotica* (Chrysomelidae: Coleoptera). Ann. Entomol. Soc. Am. 70, 168.

Figure 2.1 Photograph of single-plant emergence cage

Table 2.1 Climatic information[1] for Urbana, Illinois (2010–2012)

Year	Month	Mean temp. (°C)[2]	Cumulative heat units[3]	Cumulative heat stress units[4]	Total precip. (cm)[5]
2010	April	15 (+4)	157 (+48)	0 (0)	5.3 (–3.3)
	May	18 (+1)	407 (+82)	11 (+8)	8.7 (–4.2)
	June	24 (+2)	814 (+136)	26 (0)	21.2 (+10.6)
	July	25 (+1)	1261 (+173)	61 (+5)	9.5 (–1.9)
	August	25 (+2)	1700 (+213)	118 (+39)	4.2 (–5.9)
2011	April	12 (+1)	104 (–5)	0 (0)	18.9 (+10.3)
	May	17 (0)	314 (–11)	4 (+1)	12.5 (–0.4)
	June	23 (+1)	686 (+8)	30 (+4)	10.6 (0.0)
	July	27 (+3)	1166 (+78)	131 (+75)	4.0 (–7.4)
	August	24 (+1)	1585 (+98)	182 (+103)	4.5 (–5.6)
2012	April	12 (+1)	226 (+117)	0 (0)	5.9 (–2.7)
	May	20 (+3)	531 (+206)	25 (+22)	7.9 (–5.0)
	June	22 (0)	882 (+204)	67 (+41)	5.8 (–4.8)
	July	28 (+4)	1357 (+269)	229 (+173)	1.6 (–9.8)
	August	23 (0)	1740 (+253)	294 (+215)	14.1 (+4.0)

[1]Values in parentheses represent the difference between a reported value and the mean for that value from the 15-year period preceding this experiment (1995–2009) (Midwestern Regional Climate Center). [2]Values represent the mean daily temperature. [3]Values represent the number of cumulative heat units accumulated since the beginning of the year. Heat units were calculated using a base of 10 °C and a maximum of 30 °C. [4]Values represent the number of cumulative heat stress units accumulated since the beginning of the year. Heat stress units were calculated using a base of 30 °C. [5]Values represent the total amount of precipitation for the duration of the month.

Table 2.2 Climatic information[1] for Concord, Nebraska (2010–2012)

Year	Month	Mean temp. (°C)[2]	Cumulative heat units[3]	Cumulative heat stress units[4]	Total precip. (cm)[5]
2010	April	13 (+3)	115 (+33)	0 (–1)	7.3 (–1.5)
	May	16 (0)	292 (+18)	22 (+14)	6.4 (–4.2)
	June	22 (+1)	649 (+43)	49 (+16)	23.3 (+10.6)
	July	24 (0)	1075 (+60)	80 (–5)	21.5 (+15.0)
	August	24 (+1)	1489 (+92)	118 (+2)	5.3 (–3.5)
2011	April	8 (–2)	21 (–61)	0 (–1)	9.7 (+0.9)
	May	15 (–1)	184 (–90)	12 (+4)	13.9 (+3.3)
	June	21 (0)	497 (–109)	31 (–2)	10.1 (–2.6)
	July	26 (+2)	959 (–56)	106 (+21)	3.9 (–2.6)
	August	23 (0)	1335 (–62)	128 (+12)	15.8 (+7.0)
2012	April	12 (+2)	178 (+96)	8 (+7)	8.3 (–0.5)
	May	18 (+2)	416 (+142)	23 (+15)	13.1 (+2.5)
	June	22 (+1)	763 (+157)	59 (+26)	2.1 (–10.6)
	July	27 (+3)	1213 (+198)	207 (+122)	0.0 (–6.5)
	August	22 (–1)	1553 (+156)	274 (+158)	5.2 (–3.6)

[1]Values in parentheses represent the difference between a reported value and the mean for that value from the 15-year period preceding this experiment (1995–2009) (Midwestern Regional Climate Center). [2]Values represent the mean daily temperature. [3]Values represent the number of cumulative heat units accumulated since the beginning of the year. Heat units were calculated using a base of 10 °C and a maximum of 30 °C. [4]Values represent the number of cumulative heat stress units accumulated since the beginning of the year. Heat stress units were calculated using a base of 30 °C. [5]Values represent the total amount of precipitation for the duration of the month.

Table 2.3 Statistical tests[1] of fixed effects for node-injury ratings, consistency percentages, yields, and barren stalk percentages (2010–2012)

Variable	Year	Effect	df_N	df_D	F value	P value
Node-injury rating	2010	Site	3	11.9	4.3	0.03
		Treatment	2	24.7	9.8	< 0.01
		Site × treatment	6	24.4	4.0	< 0.01
	2011	Site	5	35.8	62.9	< 0.01
		Treatment	2	48.4	60.8	< 0.01
		Site × treatment	10	48.5	18.6	< 0.01
	2012	Site	5	20.1	127.2	< 0.01
		Treatment	2	40.0	144.0	< 0.01
		Site × treatment	10	40.1	88.1	< 0.01
Consistency percentage	2010	Site	3	6.8	1.2	0.39
		Treatment	2	33.3	2.1	0.14
		Site × treatment	6	33.3	1.8	0.13
	2011	Site	5	32.2	28.9	< 0.01
		Treatment	2	80.0	25.4	< 0.01
		Site × treatment	10	80.0	7.9	< 0.01
	2012	Site	5	26.6	58.8	< 0.01
		Treatment	2	84.0	33.6	< 0.01
		Site × treatment	10	84.0	20.3	< 0.01
Yield	2010	Site	3	10.0	24.5	< 0.01
		Treatment	2	40.3	7.3	< 0.01
		Site × treatment	6	40.3	1.3	0.29
	2011	Site	5	15.5	24.6	< 0.01
		Treatment	2	74.1	6.3	< 0.01
		Site × treatment	10	74.0	3.6	< 0.01
Barren stalk percentage	2012	Site	5	24.1	151.9	< 0.01
		Treatment	2	84.0	4.9	< 0.01
		Site × treatment	10	84.0	4.8	< 0.01

[1]Statistical tests were performed using PROC MIXED of SAS 9.2. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D). Data were analyzed using a square-root transformation.

Table 2.4 Mean[1] node-injury ratings for the interaction of site and treatment (2010)

Location	Previous crop	Treatment[2]	NIR (0–3)[3]
Illinois	Corn	Isoline	0.02 b
		Single toxin	0.01 b
		Pyramid	0.01 b
	Soybean	Isoline	0.02 b
		Single toxin	0.02 b
		Pyramid	0.01 b
Nebraska	Corn	Isoline	0.07 a
		Single toxin	0.01 b
		Pyramid	0.01 b
	Soybean	Isoline	0.01 b
		Single toxin	0.01 b
		Pyramid	0.00 b

[1]Mean comparisons were performed using PROC MIXED of SAS 9.2. Means within a column that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analyzed using a square-root transformation—actual means are reported. [2]The "isoline" treatment included hybrids without western corn rootworm-active insecticidal toxins. The "single toxin" treatment included hybrids expressing either Cry34/35Ab1 or Cry3Bb1. The "pyramid" treatment was a hybrid expressing both Cry34/35Ab1 and Cry3Bb1. [3]Values were determined using the 0 to 3 node-injury scale (Oleson et al. 2005) (NIR = node-injury rating).

Table 2.5 Mean[1] node-injury ratings, consistency percentages, and yields for the interaction of site and treatment (2011)

Location	Previous crop	Treatment[2]	NIR (0–3)[3]	Consistency (%)[4]	Yield (t/ha)[5]
Illinois	Corn	Isoline	0.94 b	33 b	7.7 g
		Single toxin	0.04 c	98 a	10.7 de
		Pyramid	0.01 c	100 a	10.0 ef
	Soybean	Isoline	0.01 c	100 a	12.0 bcd
		Single toxin	0.01 c	100 a	11.7 cd
		Pyramid	0.01 c	100 a	10.7 de
	Trap crop	Isoline	0.01 c	100 a	11.1 de
		Single toxin	0.01 c	100 a	10.7 de
		Pyramid	0.00 c	100 a	10.6 de
Nebraska	Corn	Isoline	0.14 c	88 a	13.7 a
		Single toxin	0.02 c	100 a	13.6 a
		Pyramid	0.01 c	100 a	13.3 abc
	Soybean	Isoline	0.00 c	100 a	13.4 ab
		Single toxin	0.00 c	100 a	13.5 a
		Pyramid	0.01 c	100 a	13.1 abc
	Trap crop	Isoline	1.46 a	20 b	8.0 g
		Single toxin	0.15 c	87 a	10.6 de
		Pyramid	0.19 c	87 a	8.6 fg

[1]Mean comparisons were performed using PROC MIXED of SAS 9.2. Means within a column that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analyzed using a square-root transformation—actual means are reported. [2]The "isoline" treatment included hybrids without western corn rootworm-active insecticidal toxins. The "single toxin" treatment included hybrids expressing either Cry34/35Ab1 or Cry3Bb1. The "pyramid" treatment was a hybrid expressing both Cry34/35Ab1 and Cry3Bb1. [3]Values were determined using the 0 to 3 node-injury scale (Oleson et al. 2005) (NIR = node-injury rating). [4]Values represent the percentage of root systems per plot with a node-injury rating less than 0.25. [5]Corn was harvested from the center two rows of each plot, weighed, and converted to t/ha at 15.5% moisture.

Table 2.6 Mean[1] node-injury ratings, consistency percentages, and barren stalk percentages for the interaction of site and treatment (2012)

Location	Previous crop	Treatment[2]	NIR (0–3)[3]	Consistency (%)[4]	Barren stalks (%)[5]
Illinois	Corn	Isoline	0.19 c	78 bc	74 a
		Single toxin	0.02 d	100 a	40 bc
		Pyramid	0.01 d	100 a	30 cd
	Soybean	Isoline	0.03 d	100 a	1 fg
		Single toxin	0.02 d	100 a	9 ef
		Pyramid	0.00 d	100 a	5 efg
	Trap crop	Isoline	2.04 a	3 d	78 a
		Single toxin	0.35 b	68 c	45 b
		Pyramid	0.02 d	100 a	15 de
Nebraska	Corn	Isoline	0.08 d	90 ab	94 a
		Single toxin	0.09 d	90 ab	84 a
		Pyramid	0.01 d	100 a	98 a
	Soybean	Isoline	0.01 d	100 a	0 g
		Single toxin	0.00 d	100 a	0 g
		Pyramid	0.04 d	90 ab	0 g
	Trap crop	Isoline	0.09 d	90 ab	83 a
		Single toxin	0.02 d	100 a	76 a
		Pyramid	0.00 d	100 a	100 a

[1]Mean comparisons were performed using PROC MIXED of SAS 9.2. Means within a column that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analyzed using a square-root transformation—actual means are reported. [2]The "isoline" treatment included hybrids without western corn rootworm-active insecticidal toxins. The "single toxin" treatment included hybrids expressing either Cry34/35Ab1 or Cry3Bb1. The "pyramid" treatment was a hybrid expressing both Cry34/35Ab1 and Cry3Bb1. [3]Values were determined using the 0 to 3 node-injury scale (Oleson et al. 2005) (NIR = node-injury rating). [4]Values represent the percentage of root systems per plot with a node-injury rating less than 0.25. [5]Values represent the percentage of stalks per plot without ears.

Table 2.7 Statistical tests[1] of fixed effects for cumulative emergence (2010–2012)

Variable	Year	Effect	df_N	df_D	F value	P value
Female	2010	Site	3	23.9	3.1	0.05
		Treatment	2	43.7	1.1	0.36
		Site × treatment	6	43.6	0.6	0.76
	2011	Site	5	18.9	19.3	< 0.01
		Treatment	2	80.0	19.5	< 0.01
		Site × treatment	10	80.0	4.7	< 0.01
	2012	Site	5	20.2	14.3	< 0.01
		Treatment	2	82.9	13.8	< 0.01
		Site × treatment	10	82.9	1.4	0.18
Male	2010	Site	3	31.9	1.5	0.23
		Treatment	2	43.6	0.3	0.77
		Site × treatment	6	43.5	0.6	0.77
	2011	Site	5	18.8	11.7	< 0.01
		Treatment	2	80.0	24.9	< 0.01
		Site × treatment	10	80.0	4.6	< 0.01
	2012	Site	5	20.1	15.7	< 0.01
		Treatment	2	82.5	15.9	< 0.01
		Site × treatment	10	82.5	1.9	0.06

[1]Statistical tests were performed using PROC MIXED of SAS 9.2. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D). Data were analyzed using a square-root transformation.

Table 2.8 Mean[1] cumulative emergence for the interaction of site and treatment (2011)

Location	Previous crop	Treatment[2]	Female[3]	Male[3]
Illinois	Corn	Isoline	28 a	15 a
		Single toxin	10 b	5 b
		Pyramid	4 bcd	2 cd
	Soybean	Isoline	1 de	1 d
		Single toxin	1 de	0 d
		Pyramid	0 e	0 d
	Trap crop	Isoline	8 b	5 b
		Single toxin	2 cde	2 cd
		Pyramid	2 cde	1 d
Nebraska	Corn	Isoline	2 cde	1 d
		Single toxin	0 e	0 d
		Pyramid	0 e	0 d
	Soybean	Isoline	0 e	0 d
		Single toxin	0 e	0 d
		Pyramid	0 e	0 d
	Trap crop	Isoline	5 bc	3 bc
		Single toxin	7 b	1 d
		Pyramid	4 bcd	1 d

[1]Mean comparisons were performed using PROC MIXED of SAS 9.2. Means within a column that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analyzed using a square-root transformation—actual means are reported. [2]The "isoline" treatment included hybrids without western corn rootworm-active insecticidal toxins. The "single toxin" treatment included hybrids expressing either Cry34/35Ab1 or Cry3Bb1. The "pyramid" treatment was a hybrid expressing both Cry34/35Ab1 and Cry3Bb1. [3]Values represent the cumulative number of beetles captured from three single-plant emergence cages per plot throughout the growing season.

Table 2.9 Statistical tests[1] of fixed effects for female-to-male sex ratios (2010–2012)

Year	Effect	df_N	df_D	F value	P value
2010	Site	—	—	—	—
	Treatment	—	—	—	—
	Site × treatment	—	—	—	—
2011	Site	2	13.5	1.2	0.34
	Treatment	2	31.2	0.3	0.77
	Site × treatment	4	31.1	1.0	0.44
2012	Site	4	16.8	3.6	0.03
	Treatment	2	61.5	6.0	< 0.01
	Site × treatment	8	59.9	1.2	0.30

[1]Statistical tests were performed using PROC MIXED of SAS 9.2. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D). Data were analyzed using a square-root transformation. Values represented by a dash were nonestimable.

Table 2.10 Statistical tests[1] of the fixed effect of treatment for female head capsule widths (2010–2012)

Year	Location	Previous crop	df_N	df_D	F value	P value
2010	Illinois	Corn	2	3.8	21.7	0.01
		Soybean	2	3.0	0.2	0.83
	Nebraska	Corn	—	—	—	—
		Soybean	—	—	—	—
2011	Illinois	Corn	2	14.0	0.7	0.51
		Soybean	1	1.3	< 0.1	0.95
		Trap crop	2	10.7	1.4	0.29
	Nebraska	Corn	1	5.2	0.1	0.77
		Soybean	—	—	—	—
		Trap crop	2	9.1	0.3	0.75
2012	Illinois	Corn	2	14.0	5.0	0.02
		Soybean	2	14.0	7.3	0.01
		Trap crop	2	14.0	4.6	0.03
	Nebraska	Corn	2	10.8	1.0	0.40
		Soybean	—	—	—	—
		Trap crop	2	13.5	1.2	0.32

[1]Statistical tests were performed using PROC MIXED of SAS 9.2. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D). Data were analyzed using a square-root transformation. Values represented by a dash were nonestimable.

Table 2.11 Mean[1] head capsule widths and weights for the effect of treatment (2010)

			Head capsule width (μm)		Weight (mg)[3]	
Location	Previous crop	Treatment[2]	Female	Male	Female	Male
Illinois	Corn	Isoline	1190 b	—	2.8 a	—
		Single toxin	1140 b	—	3.3 a	—
		Pyramid	1390 a	—	1.0 a	—
	Soybean	Isoline	1220 a	—	2.4 a	—
		Single toxin	1190 a	—	2.9 a	—
		Pyramid	1200 a	—	4.0 a	—
Nebraska	Corn	Isoline	—	—	—	—
		Single toxin	—	—	—	—
		Pyramid	—	—	—	—
	Soybean	Isoline	—	—	—	—
		Single toxin	—	—	—	—
		Pyramid	—	—	—	—

[1]Mean comparisons were performed using PROC MIXED of SAS 9.2. Means for an individual site (i.e., a specific combination of location and previous crop) that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analyzed using a square-root transformation—actual means are reported. Values represented by a dash were nonestimable. [2]The "isoline" treatment included hybrids without western corn rootworm-active insecticidal toxins. The "single toxin" treatment included hybrids expressing either Cry34/35Ab1 or Cry3Bb1. The "pyramid" treatment was a hybrid expressing both Cry34/35Ab1 and Cry3Bb1. [3]Beetles were dried for 24 h at 60 °C prior to weighing to ensure a uniform moisture concentration.

Table 2.12 Mean[1] head capsule widths and weights for the effect of treatment (2011)

			Head capsule width (μm)		Weight (mg)[3]	
Location	Previous crop	Treatment[2]	Female	Male	Female	Male
Illinois	Corn	Isoline	1110 a	1110 a	2.1 a	2.1 a
		Single toxin	1090 a	1100 a	2.3 a	1.9 a
		Pyramid	1100 a	1030 a	2.0 a	1.4 a
	Soybean	Isoline	1130 a	—	2.6 a	—
		Single toxin	1160 a	—	3.1 a	—
		Pyramid	—	—	—	—
	Trap crop	Isoline	1160 a	1130 a	3.4 a	2.6 a
		Single toxin	1090 a	1100 a	2.9 a	1.7 b
		Pyramid	1170 a	1130 a	3.7 a	2.0 ab
Nebraska	Corn	Isoline	1130 a	1140 a	2.1 a	2.0 a
		Single toxin	1110 a	1150 a	2.3 a	1.4 b
		Pyramid	—	—	—	—
	Soybean	Isoline	—	—	—	—
		Single toxin	—	—	—	—
		Pyramid	—	—	—	—
	Trap crop	Isoline	1040 a	1090 a	1.9 a	1.7 a
		Single toxin	1060 a	1030 a	1.9 a	1.5 a
		Pyramid	1070 a	1070 a	2.3 a	1.4 a

[1]Mean comparisons were performed using PROC MIXED of SAS 9.2. Means for an individual site (i.e., a specific combination of location and previous crop) that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analyzed using a square-root transformation—actual means are reported. Values represented by a dash were nonestimable. [2]The "isoline" treatment included hybrids without western corn rootworm-active insecticidal toxins. The "single toxin" treatment included hybrids expressing either Cry34/35Ab1 or Cry3Bb1. The "pyramid" treatment was a hybrid expressing both Cry34/35Ab1 and Cry3Bb1. [3]Beetles were dried for 24 h at 60 °C prior to weighing to ensure a uniform moisture concentration.

Table 2.13 Mean[1] head capsule widths and weights for the effect of treatment (2012)

			Head capsule width (μm)		Weight (mg)[3]	
Location	Previous crop	Treatment[2]	Female	Male	Female	Male
Illinois	Corn	Isoline	1170 a	1170 a	2.6 a	2.6 a
		Single toxin	1140 b	1090 b	3.0 a	2.2 a
		Pyramid	1120 b	1040 c	3.0 a	2.4 a
	Soybean	Isoline	1240 a	1200 a	2.7 ab	2.4 a
		Single toxin	1190 b	1160 a	2.5 b	2.1 a
		Pyramid	1160 b	1090 b	3.3 a	1.4 b
	Trap crop	Isoline	1140 a	1140 a	2.1 a	2.1 a
		Single toxin	1100 ab	1110 a	1.8 a	1.8 ab
		Pyramid	1070 b	1100 a	2.4 a	1.5 b
Nebraska	Corn	Isoline	1190 a	1190 a	3.0 a	2.7 a
		Single toxin	1170 a	1150 a	3.6 a	3.0 a
		Pyramid	1140 a	1050 b	3.8 a	3.2 a
	Soybean	Isoline	—	—	—	—
		Single toxin	—	—	—	—
		Pyramid	—	—	—	—
	Trap crop	Isoline	1150 a	1160 a	2.3 a	2.2 a
		Single toxin	1120 a	1140 a	2.5 a	1.7 ab
		Pyramid	1120 a	1110 a	2.3 a	1.3 b

[1]Mean comparisons were performed using PROC MIXED of SAS 9.2. Means for an individual site (i.e., a specific combination of location and previous crop) that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analyzed using a square-root transformation—actual means are reported. Values represented by a dash were nonestimable. [2]The "isoline" treatment included hybrids without western corn rootworm-active insecticidal toxins. The "single toxin" treatment included hybrids expressing either Cry34/35Ab1 or Cry3Bb1. The "pyramid" treatment was a hybrid expressing both Cry34/35Ab1 and Cry3Bb1. [3]Beetles were dried for 24 h at 60 °C prior to weighing to ensure a uniform moisture concentration.

Table 2.14 Statistical tests[1] of the fixed effect of treatment for male head capsule widths (2010–2012)

Year	Location	Previous crop	df_N	df_D	F value	P value
2010	Illinois	Corn	—	—	—	—
		Soybean	—	—	—	—
	Nebraska	Corn	—	—	—	—
		Soybean	—	—	—	—
2011	Illinois	Corn	2	3.7	4.2	0.11
		Soybean	—	—	—	—
		Trap crop	2	9.2	0.8	0.50
	Nebraska	Corn	1	2.9	< 0.1	0.95
		Soybean	—	—	—	—
		Trap crop	2	3.6	1.4	0.35
2012	Illinois	Corn	2	12.9	20.6	< 0.01
		Soybean	2	14.0	10.3	< 0.01
		Trap crop	2	13.9	2.9	0.09
	Nebraska	Corn	2	10.1	6.0	0.02
		Soybean	—	—	—	—
		Trap crop	2	7.3	2.2	0.18

[1]Statistical tests were performed using PROC MIXED of SAS 9.2. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D). Data were analyzed using a square-root transformation. Values represented by a dash were nonestimable.

Table 2.15 Statistical tests[1] of the fixed effect of treatment for female weights (2010–2012)

Year	Location	Previous crop	df_N	df_D	F value	P value
2010	Illinois	Corn	2	2.0	6.3	0.27
		Soybean	2	5.0	1.9	0.25
	Nebraska	Corn	—	—	—	—
		Soybean	—	—	—	—
2011	Illinois	Corn	2	14.0	2.3	0.14
		Soybean	1	2.0	< 0.1	0.94
		Trap crop	2	10.1	0.9	0.45
	Nebraska	Corn	1	6.4	0.3	0.61
		Soybean	—	—	—	—
		Trap crop	2	9.1	0.8	0.48
2012	Illinois	Corn	2	14.0	1.5	0.26
		Soybean	2	14.0	4.0	0.04
		Trap crop	2	14.0	2.1	0.17
	Nebraska	Corn	2	10.0	1.2	0.35
		Soybean	—	—	—	—
		Trap crop	2	13.3	0.3	0.72

[1]Statistical tests were performed using PROC MIXED of SAS 9.2. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D). Data were analyzed using a square-root transformation. Values represented by a dash were nonestimable.

Table 2.16 Statistical tests[1] of the fixed effect of treatment for male weights (2010–2012)

Year	Location	Previous crop	df_N	df_D	F value	P value
2010	Illinois	Corn	—	—	—	—
		Soybean	—	—	—	—
	Nebraska	Corn	—	—	—	—
		Soybean	—	—	—	—
2011	Illinois	Corn	2	13.4	3.2	0.07
		Soybean	—	—	—	—
		Trap crop	2	10.9	7.9	0.01
	Nebraska	Corn	1	1.0	175.2	0.05
		Soybean	—	—	—	—
		Trap crop	2	5.6	0.4	0.70
2012	Illinois	Corn	2	13.5	1.1	0.37
		Soybean	2	14.0	17.6	< 0.01
		Trap crop	2	13.9	3.8	0.05
	Nebraska	Corn	2	9.8	0.7	0.51
		Soybean	—	—	—	—
		Trap crop	2	7.1	5.1	0.04

[1]Statistical tests were performed using PROC MIXED of SAS 9.2. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D). Data were analyzed using a square-root transformation. Values represented by a dash were nonestimable.

CHAPTER 3: EXAMINATION OF POTENTIAL DIFFERENCES BETWEEN ROTATION-RESISTANT AND ROTATION-SUSCEPTIBLE POPULATIONS OF THE WESTERN CORN ROOTWORM USING A GREENHOUSE BIOASSAY

Introduction

Corn, *Zea mays* (L.), is an important grain crop cultivated globally for a variety of uses. The United States accounted for approximately 20% of the 170 million ha of corn planted worldwide in 2012 (USDA FAS 2012). One of the most economically important insect pests threatening corn production in the United States is the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (Coleoptera: Chrysomelidae). Yield losses and control costs associated with corn rootworm species—which include the northern (*Diabrotica barberi* Smith & Lawrence) and southern (*Diabrotica undecimpunctata howardi* Barber) corn rootworms as well—exceed $1 billion annually in the United States (Metcalf 1986). Western corn rootworm larvae feed on the root tissue of corn plants, which can result in impaired photosynthesis (Godfrey et al. 1993; Riedell and Reese 1999); reduced uptake of water (Godfrey et al. 1993) and nitrogen (Spike and Tollefson 1989); colonization by phytopathogenic or saprophytic fungi (Bryson et al. 1953; Kurtz et al. 2010); and plant lodging (Spike and Tollefson 1991). Although yield loss due to root injury is highly variable (Gray and Steffey 1998), Tinsley et al. (2013) estimated that for every node (i.e., circle of roots) consumed by corn rootworm larvae, a yield loss of approximately 15% can be expected.

Throughout its history as a pest of corn, the western corn rootworm has demonstrated a high degree of adaptability, as it has overcome a variety of pest management strategies (Gray et al. 2009). This insect has evolved resistance or reduced susceptibility to carbamate (Meinke et al. 1998), organophosphate (Ball 1968; Call et al. 1977), and organochlorine (Ball and Weekman 1962; Hamilton 1965) insecticides. Crop rotation (rotating corn with a non-host crop) was widely successful for managing the western corn rootworm since it was first recommended in the early 1900s (Gillette 1912). However, much of the east-central Corn Belt now hosts a variant western corn rootworm with resistance to crop rotation (Levine et al. 2002). Additionally, resistance to genetically modified corn expressing an insecticidal toxin (Cry3Bb1) from *Bacillus thuringiensis* Berliner (Bt) has been confirmed for this insect in Iowa (Gassmann et al. 2011) and Illinois (Gray 2012). Unfortunately, little information is available concerning the potential interaction of adaptive populations of the western corn rootworm with alternate

management tactics. One example of this type of interaction is the relative effectiveness of Bt toxins against rotation-resistant and rotation-susceptible populations of the western corn rootworm. Understanding this type of interaction may allow applied entomologists to refine their management recommendations.

Gray et al. (2007) evaluated a number of Bt hybrids targeting the western corn rootworm—all hybrids expressed the same Bt toxin (Cry3Bb1), which was inserted using the same transgenic event (MON-ØØ863-5). During one year of their experiment (2006), two trial sites were evaluated (Monmouth and Urbana, IL). Urbana is located in a region of Illinois that has historically had high populations of the rotation-resistant western corn rootworm; however, at Monmouth, rotation-resistant populations of this pest were much lower. Although root injury for the non-Bt untreated check was similar at both locations, root injury for the Bt hybrids was approximately 105% greater at the Urbana site. As a result, the authors hypothesized that rotation-resistant and rotation-susceptible larvae may differ in their capacity to injure roots. However, the experimental design used by Gray et al. (2007) was not constructed in a manner that could test this hypothesis explicitly—Bt hybrids at Monmouth and Urbana may have been subjected to feeding by both rotation-resistant and rotation-susceptible larvae. The goal of the greenhouse experiment described herein was to determine if rotation-resistant and rotation-susceptible western corn rootworm larvae differ with respect to survival or development when exposed to corn expressing Bt toxins. A single-plant bioassay was used with techniques adapted from Gassmann et al. (2011).

Materials and Methods

Experimental Design

This experiment was conducted during 2012 and 2013 using a two-way factorial arrangement in a completely randomized design. The first factor was western corn rootworm population and included two levels: (1) rotation-resistant and (2) rotation-susceptible. For the remainder of this chapter, this first factor will be referred to as "population." Larvae from each population were evaluated for survival and development on five corn hybrids, including a hybrid expressing Cry34/35Ab1 (Event DAS-59122-7, Pioneer 34P94, DuPont Pioneer, Johnston, IA), its near-isoline (Pioneer 34P89, DuPont Pioneer), a hybrid expressing Cry3Bb1 (Event MON-88Ø17-3, DKC61-19,

Monsanto Co., St. Louis, MO), its near-isoline (DKC61-22, Monsanto Co.), and a hybrid expressing both Cry34/35Ab1 and Cry3Bb1 (Events DAS-59122-7 and MON-88Ø17-3, DKC61-21, Monsanto Co.)—DKC61-19 and DKC61-21 shared a common near-isoline (DKC61-22). To simplify analyses of the second factor, hybrids were pooled to create three levels: (1) an "isoline" treatment that included hybrids without western corn rootworm-active insecticidal toxins, (2) a "single toxin" treatment that included hybrids expressing either Cry34/35Ab1 or Cry3Bb1, and (3) a "pyramid" treatment that included the hybrid expressing both Cry34/35Ab1 and Cry3Bb1. For the remainder of this chapter, this second factor will be referred to as "treatment." In total, three runs of this experiment were performed. For each run, four replications of each population × treatment combination were used.

Seed Preparation and Planting Information

All seed used during this experiment was treated with a neonicotinoid insecticidal seed treatment (either clothianidin or thiamethoxam) at 0.25 mg active ingredient/seed. These insecticidal treatments were removed to avoid confounding any mortality due to the insecticides with mortality caused by the Bt toxins being evaluated during this experiment. Seeds were first placed in a 1% detergent solution (Dawn Ultra Concentrated Dish Soap, Proctor & Gamble, Cincinnati, OH) and agitated gently for 20 min. Following agitation, the seeds were rinsed thoroughly under running water. This wash/rinse procedure was repeated for a total of three cycles. Washed seeds were allowed to dry for 12 h, after which they were soaked in a 10% bleach solution (Clorox Regular Bleach, The Clorox Co., Oakland, CA). While soaking in the bleach solution, the seeds were stirred periodically. After soaking for approximately 1 h, the seeds were rinsed thoroughly under running water and allowed to dry for at least 24 h prior to planting.

This experiment was conducted at the Natural Resources Studies Annex Greenhouse at Champaign, IL. The greenhouse was maintained at 25 °C with 16 h of light each day. For each run, 40 single-plant pots were prepared (2 populations × 5 hybrids × 4 replications/run). Pots were made of white polypropylene and had a volume of 950 ml (Item No. 81144, US Plastic Corp., Lima, OH). Four 3 mm holes were drilled into the bottom of each pot for drainage. The pots were then filled with 750 ml of a 1:1 mixture of potting soil (Sunshine Mix LC1, Sun Gro Horticulture Canada Ltd., Vancouver, BC) and sterilized topsoil. Prior to planting the seeds, each pot was fertilized

with 4 g of a 14-14-14 (N-P-K) slow-release fertilizer (Osmocote Smart-Release Plant Food, The Scotts Miracle-Gro Co., Marysville, OH). A single seed was planted in the center of each pot at a depth of 1 cm. Pots were watered as needed to maintain a uniform level of moisture, with a maximum of 50 ml of water per day. Throughout this experiment, plants were periodically trimmed so that their overall height did not exceed 30 cm—trimming the plants reduced their overall water requirement and helped maintain consistent moisture.

Egg Preparation and Infestation Technique

The western corn rootworm eggs used during this experiment were provided by personnel at the USDA ARS North Central Agricultural Research Laboratory (NCARL) at Brookings, SD. The rotation-resistant and rotation-susceptible populations were identified as "Spencer" and "Whitlock," respectively. Upon receipt, the eggs and their soil matrix were soaked in water for approximately 30 min. To separate eggs from the soil, the solution was poured through a stainless steel mesh sieve with 250 µm pores. The eggs were rinsed under running water for 5 min to remove all soil. The eggs were then placed into a 950 ml container filled with 750 ml of water. The eggs were examined regularly to monitor their development. To prevent fungal growth in the egg containers, approximately 75% of the water was replaced daily. Eggs were ready to hatch (i.e., neonate larvae were visible within the eggs) after approximately 21 d of storage at room temperature.

Infestation of greenhouse plants by near-hatch eggs was timed to coincide with the V5 growth stage (five leaf collar). For each plant that needed to be infested, 225 eggs (measured volumetrically) were transferred from the egg containers into a 1.5 ml microcentrifuge tube. To infest each plant, a 10 cm-long hole was bored into the soil approximately 2.5 cm from the base of the plant. The pre-measured volume of eggs was transferred from the microcentrifuge tube to the hole using a plastic pipette. After depositing the eggs, the infestation site was closed by gently pinching together the surface of the soil near the top of the hole. Larvae were allowed to develop undisturbed on each plant for 17 d—Gassmann et al. (2011) noted that this duration was sufficient to allow the fastest-developing larvae to reach the final developmental stage (instar) prior to pupation.

Larval Extraction and Processing

To extract larvae from each pot, plants were cut near the soil surface and the pots were transported to the Agricultural Engineering Farm near Urbana, IL. The contents of each pot (soil and root tissue) were gently removed

and placed onto a square piece of aluminum screen (20 cm on each side) with 45 holes/cm^2. This screen was then placed into the upper chamber of a collapsible Berlese funnel trap (Item No. 2832, BioQuip Products Inc., Rancho Dominguez, CA). These traps are commonly used to extract insects and other arthropods from leaf litter or soil and operate by utilizing a heat source (e.g., an incandescent light bulb) placed above the sample to stimulate mobile organisms to move away from the heat and toward a collection container at the bottom of the trap. For this experiment, the collection container was a Whirl-Pak bag (Item No. 1114B, BioQuip Products Inc.) filled with 50 ml of 85% ethanol. Larvae were extracted continuously for a period of 72 h, after which the number of larvae present in each sample was recorded.

From each sample, a subsample of three larvae were selected at random for head capsule measurement. Western corn rootworm larvae develop through three instars—these developmental stages can be differentiated by measuring the width of their head capsule. First, second, and third instar larvae have head capsule widths measuring within the ranges of 0–270, 270–410, and 410–580 µm, respectively (Hammack et al. 2003). Head capsule widths were determined for each larva by measuring the widest portion of the head. A dissecting microscope (Model MZ125, Leica Microsystems Inc., Buffalo Grove, IL) with an ocular reticle was used—a magnification of 10X was selected to achieve an accuracy of 10 µm.

Statistical Analysis

To standardize comparisons between western corn rootworm populations, the relative corrected mortality for each single toxin and pyramid sample was determined using formulae adapted from Abbott (1925). This metric is "relative" because it is calculated for each population independently and "corrected" because it accounts for natural mortality not caused by exposure to Bt toxins. First, the proportional survival for each sample was calculated using the formula $s_{ti} = l_{ti} \div r$ where s_{ti} = the proportional survival for sample i of treatment t, l_{ti} = the number of larvae recovered from sample i of treatment t, and r = the infestation rate. Next, the mean proportional survival for the isoline treatment for each population was calculated using the formula $\bar{s}_t = \left(\sum_{i=1}^{n_t}[s_{ti}]\right) \div n_t$ where $\bar{s}_t$ = the mean proportional survival for the isoline treatment t, s_{ti} = the proportional survival for sample i of the isoline treatment t, and n_t = the total number of samples for the isoline treatment t. Finally, the corrected mortality for each sample of the single toxin and pyramid treatments for each population

was calculated using the formula $m_{ti} = ([\bar{s}_t - s_{ti}] \div \bar{s}_t) \times 100$ where m_{ti} = the corrected mortality for sample i of the single toxin or pyramid treatment t, $\bar{s}_t$ = the mean proportional survival for the isoline treatment t, and s_{ti} = the proportional survival for sample i of the single toxin or pyramid treatment t.

Response variables (proportional survival on the isoline treatment, relative corrected mortality on the single toxin and pyramid treatments, and head capsule widths) were analyzed using SAS 9.2 (SAS Institute Inc., Cary, NC). To stabilize variances and meet the assumptions of analysis of variance, response variables were analyzed using a square-root transformation. Statistical tests for fixed effects were performed using PROC MIXED. Fixed effects included population, treatment, and their interaction and were declared significant at $P \leq 0.05$. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D) (Kenward and Roger 1997). Means were compared using the LSMEANS option of PROC MIXED and were declared significantly different at $P \leq 0.05$.

Results

Mean proportional survival was only evaluated for the isoline treatment. As a result, only the effect of population was included in the analysis for this response variable. A significant effect of population on mean proportional survival on the isoline treatment was observed (Table 3.1). Mean proportional survival on the isoline treatment for rotation-resistant and rotation-susceptible larvae differed significantly—rotation-resistant larvae averaged a 14.0% greater rate of proportional survival than rotation-susceptible larvae (Table 3.2).

No significant effect of population, treatment, or their interaction was observed for mean relative corrected mortality (Table 3.3). With respect to the effect of population, mean relative corrected mortality rates for rotation-resistant (68.5%) and rotation-susceptible (71.4%) larvae were statistically similar. For the effect of treatment, mean relative corrected mortality rates for larvae on the single toxin (69.0%) and pyramid (71.8%) treatments were not significantly different. For the interaction of population and treatment, mean relative corrected mortality rates ranged from 67.0 to 72.0% and were statistically similar for all population × treatment combinations (Table 3.4).

Only the effect of treatment on mean head capsule widths was statistically significant—the effect of population and the interaction of treatment and population were not (Table 3.5). With respect to the effect of population, mean head capsule widths for rotation-resistant (245 µm) and rotation-susceptible (240 µm) larvae were statistically similar. For the effect of treatment, the mean head capsule width for larvae on the isoline treatment (265 µm) was significantly greater than the mean head capsule widths for larvae on the single toxin (231 µm) and pyramid (222 µm) treatments, which were statistically similar. Mean head capsule widths with respect to the interaction of population and treatment are reported in Table 3.6.

Discussion

Proportional survival of larvae on the isoline treatment was analyzed to determine if the level of natural mortality differed between the two populations of western corn rootworm evaluated during this experiment. Determining proportional survival for each population was necessary to standardize comparisons of mortality caused by treatments containing Bt toxins. Because proportional survival between rotation-resistant and rotation-susceptible larvae differed significantly (Table 3.2), the decision to use a relative parameter to analyze corrected mortality caused by the Bt toxins was justified. It is unclear why these two populations differed with respect to proportional survival on the isoline treatment. The rotation-resistant population was represented by the Spencer colony, which has been in culture since 2010 (ca. 2 generations). In contrast, the Whitlock colony was used to represent the rotation-susceptible population—this colony has been in culture since 1996 (ca. 16 generations) (C. N. Nielson, USDA ARS NCARL, personal communication). Although the duration of time spent in culture between these two colonies varies substantially, it is unlikely that this difference contributed to the observed difference in proportional survival. Kim et al. (2007) used microsatellite loci to compare genetic diversity among various colonies maintained at USDA ARS NCARL (including some wild type populations as well). Their findings demonstrated that western corn rootworm colonies maintained in culture for up to 22 generations could not be differentiated from wild type populations based on genetic diversity.

One of the principal benefits associated with Bt corn is its demonstrated ability to cause substantial mortality among western corn rootworm larvae (Storer et al. 2006; Hibbard et al. 2010). During this experiment, relative

corrected mortality was analyzed to determine if rotation-resistant and rotation-susceptible larvae differed with respect to survival on Bt corn. I failed to observe a significant effect of population on relative corrected mortality (Table 3.3), suggesting that mortality caused by the Bt toxins evaluated during this experiment (Cry34/35Ab1 and Cry3Bb1) does not differ between rotation-resistant and rotation-susceptible larvae. Additionally, no significant effect of treatment on relative corrected mortality was observed (Table 3.3)—larvae exposed to either the pyramid or single toxin treatments experienced similar mean rates of relative corrected mortality. This observation suggests that larval mortality caused by each of the toxins expressed in the pyramid treatment was non-additive. Results from the field experiment presented in Chapter 2 of this dissertation generally support this observation. Mean node-injury ratings for the pyramid treatment were superior to the single toxin treatment in only one instance (Illinois C/T, 2012, Table 2.6).

The demonstration of non-additive larval mortality associated with the pyramid treatment may have implications for integrated resistance management. Onstad and Meinke (2010) suggested that the evolution of resistance to Bt corn by the western corn rootworm would be delayed if a pyramided, multiple-toxin strategy was adopted over a single-toxin approach. However, Onstad et al. (2011) questioned the value of a pyramid strategy for integrated resistance management if multiple-toxin Bt corn failed to increase mortality in the target pest. Recent estimates suggest that mortality caused by one such pyramided product—marketed as Genuity SmartStax (Monsanto Co.) or SmartStax (Dow AgroSciences LLC, Indianapolis, IN)—is not substantially greater than either of its constituent single toxins (Cry34/35Ab1 and Cry3Bb1) (USEPA OPPTS 2011).

Many experiments have documented delayed emergence of western corn rootworm adults when exposed to Bt corn (Storer et al. 2006; Murphy et al. 2010; Spencer et al. 2012), which has generally been attributed to slower than typical larval development. Larval head capsule widths were evaluated for a subsample of all larvae recovered during this experiment to determine if rotation-resistant and rotation-susceptible larvae differed with respect to development on Bt corn. For both rotation-resistant and rotation-susceptible larvae, mean head capsule widths were greatest for larvae on the isoline treatment. This observation agrees with results presented by Hibbard et al. (2010), which demonstrated that larvae exposed to non-Bt corn typically had greater head capsule widths than those exposed to Bt corn. Mean head capsule widths for larvae from both populations were statistically similar for

the single toxin and pyramid treatments (Table 3.6), indicating that larvae from these two populations develop similarly when reared on Bt corn.

Based on the results of their field experiment, Gray et al. (2007) hypothesized that rotation-resistant western corn rootworm larvae may cause more injury to Bt corn than rotation-susceptible larvae. During the experiment described in this chapter, mortality and development were evaluated for larvae from rotation-resistant and rotation-susceptible populations. Because mean relative corrected mortality and head capsule widths were similar for both rotation-resistant and rotation-susceptible larvae on Bt corn, I conclude that any difference in the ability of these two populations to injure roots expressing one or more Bt toxins is unrelated to survival or development.

The fundamental difference between rotation-resistant and rotation-susceptible populations of the western corn rootworm is the loss of ovipositional fidelity to cornfields among rotation-resistant females (Rondon and Gray 2004), which Pierce and Gray (2006) attributed to long-term, strict annual rotation of corn and soybean. To date, the use of genetic techniques to differentiate between rotation-resistant and rotation-susceptible beetles has been unsuccessful (Miller et al. 2006). However, Garabagi et al. (2008) noted that expression of cyclic GMP-dependent protein kinases is 25% greater in rotation-resistant females when compared with rotation-susceptible females. Genes for these proteins have been implicated in the regulation of behavior in many animals. Recently, Curzi et al. (2012) reported that digestive enzymes play a role in facilitating rotation-resistance. The authors noted that rotation-resistant females had increased constitutive expression of cathepsin L-like proteases, allowing them to overcome soybean defenses (cysteine protease inhibitors) and forage for a longer duration in soybean than rotation-susceptible females. Perhaps these changes in rotation-resistant adult behavior or physiology have no correlation with the capacity of larvae to injure corn roots.

The greenhouse experiment presented in this chapter focused on western corn rootworm larval survival and development, not potential to injure corn roots. Although no significant differences in survival or development on Bt corn between rotation-resistant and rotation-susceptible larvae were documented, other factors should be explored prior to concluding that no difference in capacity to injure corn roots exists between these two populations. One such example may be behavioral differences in root feeding. Clark et al. (2006) noted that western corn rootworm larvae exposed to Bt corn display two types of feeding behavior—sedentary or roaming. Their findings suggest that larval feeding behavior associated with exposure to Bt corn is complex. Further research

is needed to identify which factors may contribute to a potential difference in the capacity to injure corn roots between rotation-resistant and rotation-susceptible larvae.

References

Abbott WS, 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Ball HJ, 1968. A five-year study of potential western corn rootworm resistance to diazinon and phorate in Nebraska. J. Econ. Entomol. 61, 496–498.

Ball HJ, Weekman GT, 1962. Insecticide resistance in the adult western corn rootworm in Nebraska. J. Econ. Entomol. 55, 439–441.

Bryson HR, Wilbur DA, Burkhardt CC, 1953. The western corn rootworm, *Diabrotica virgifera* Lec. in Kansas. J. Econ. Entomol. 46, 995–999.

Call DJ, Walgenbach DD, Sutter GR, 1977. Corn rootworm cholinesterase activity as influenced by species, sex, age, and cold storage. J. Econ. Entomol. 70, 533–535.

Clark PL, Vaughn TT, Meinke LJ, Molina-Ochoa J, Foster JE, 2006. *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) larval feeding behavior on transgenic maize (MON 863) and its isoline. J. Econ. Entomol. 99, 722–727.

Curzi MJ, Zavala JA, Spencer JL, Seufferheld MJ, 2012. Abnormally high digestive enzyme activity and gene expression explain the contemporary evolution of a *Diabrotica* biotype able to feed on soybeans. Ecol. Evol. 2, 2005–2017.

Garabagi F, French BW, Schaafsma AW, Pauls KP, 2008. Increased expression of a cGMP-dependent protein kinase in rotation-adapted western corn rootworm (*Diabrotica virgifera virgifera* L.). Insect Biochem. Molec. 38, 697–704.

Gassmann AJ, Petzold-Maxwell JL, Keweshan RS, Dunbar MW, 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLOS ONE 6, E22629.

Gillette CP, 1912. *Diabrotica virgifera* Lec. as a corn root-worm. J. Econ. Entomol. 5, 364–366.

Godfrey LD, Meinke LJ, Wright RJ, 1993. Effects of larval injury by western corn rootworm (Coleoptera: Chrysomelidae) on gas exchange parameters of field corn. J. Econ. Entomol. 86, 1546–1556.

Gray ME, 2012. Continuing evolution confirmed of field resistance to Cry3Bb1 in some Illinois fields by western corn rootworm. In: The Bulletin: Pest Management and Crop Development Information for Illinois, Issue No. 20. University of Illinois Extension, Urbana, IL, p. 120.

Gray ME, Sappington TW, Miller NJ, Moeser J, Bohn MO, 2009. Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54, 303–321.

Gray ME, Steffey KL, 1998. Corn rootworm (Coleoptera: Chrysomelidae) larval injury and root compensation of 12 maize hybrids: an assessment of the economic injury index. J. Econ. Entomol. 91, 723–740.

Gray ME, Steffey KL, Estes RE, Schroeder JB, 2007. Responses of transgenic maize hybrids to variant western corn rootworm larval injury. J. Appl. Entomol. 131, 386–390.

Hamilton EW, 1965. Aldrin resistance in corn rootworm beetles. J. Econ. Entomol. 58, 297–300.

Hammack L, Ellsbury MM, Roehrdanz RL, Pikul Jr. JL, 2003. Larval sampling and instar determination in field populations of northern and western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 96, 1153–1159.

Hibbard BE, Clark TL, Ellersieck MR, Meihls LN, El Khishen AA, Kaster V, Steiner H, Kurtz R, 2010. Mortality of western corn rootworm larvae on MIR604 transgenic maize roots: field survivorship has no significant impact on survivorship of F1 progeny on MIR604. J. Econ. Entomol. 103, 2187–2196.

Kenward MG, Roger JH, 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53, 983–997.

Kim SK, French BW, Sumerford DV, Sappington TW, 2007. Genetic diversity in laboratory colonies of western corn rootworm (Coleoptera: Chrysomelidae), including a nondiapause colony. Environ. Entomol. 36, 637–645.

Kurtz B, Karlovsky P, Vidal S, 2010. Interaction between western corn rootworm (Coleoptera: Chrysomelidae) larvae and root-infecting *Fusarium verticillioides*. Environ. Entomol. 39, 1532–1538.

Levine E, Spencer JL, Isard SA, Onstad DW, Gray ME, 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Am. Entomol. 48, 94–107.

Meinke LJ, Siegfried BD, Wright RJ, Chandler LD, 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J. Econ. Entomol. 91, 594–600.

Metcalf RL, 1986. Foreword. In: Methods for the Study of Pest *Diabrotica*, ed. Krysan JL, Miller TA. Springer, New York, NY, pp. vii–xv.

Miller NJ, Kim KS, Ratcliffe ST, Estoup A, Bourguet D, Guillemaud T, 2006. Absence of genetic divergence between western corn rootworms (Coleoptera: Chrysomelidae) resistant and susceptible to control by crop rotation. J. Econ. Entomol. 99, 685–690.

Murphy AF, Ginzel MD, Krupke CH, 2010. Evaluating western corn rootworm (Coleoptera: Chrysomelidae) emergence and root damage in a seed mix refuge. J. Econ. Entomol. 103, 147–157.

Onstad DW, Meinke LJ, 2010. Modeling evolution of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) to transgenic corn with two insecticidal traits. J. Econ. Entomol. 103, 849–860.

Onstad DW, Mitchell PD, Hurley TM, Lundgren JG, Porter RP, Krupke CH, Spencer JL, DiFonzo CD, Baute TS, Hellmich RL, Buschman LL, Hutchison WD, Tooker JF, 2011. Seeds of change: corn seed mixtures for resistance management and integrated pest management. J. Econ. Entomol. 104, 343–352.

Pierce CMF, Gray ME, 2006. Western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), oviposition: a variant's response to maize phenology. Environ. Entomol. 35, 423–434.

Riedell WE, Reese RN, 1999. Maize morphology and shoot CO_2 assimilation after root damage by western corn rootworm larvae. Crop Sci. 39, 1332–1340.

Rondon SI, Gray ME, 2004. Ovarian development and ovipositional preference of the western corn rootworm (Coleoptera: Chrysomelidae) variant in east central Illinois. J. Econ. Entomol. 97, 390–396.

Spencer J, Onstad D, Krupke C, Hughson S, Pan Z, Stanley B, Flexner L, 2012. Isolated females and limited males: evolution of insect resistance in structured landscapes. Entomol. Exp. Appl. 146, 38–49.

Spike BP, Tollefson JJ, 1989. Relationship of plant phenology to corn yield loss resulting from western corn rootworm (Coleoptera: Chrysomelidae) larval injury, nitrogen deficiency, and high plant density. J. Econ. Entomol. 82, 226–231.

Spike BP, Tollefson JJ, 1991. Yield response of corn subjected to western corn rootworm (Coleoptera: Chrysomelidae) infestation and lodging. J. Econ. Entomol. 84, 1585–1590.

Storer NP, Babcock JM, Edwards JM, 2006. Field measures of western corn rootworm (Coleoptera: Chrysomelidae) mortality caused by Cry34/35Ab1 proteins expressed in maize event 59122 and implications for trait durability. J. Econ. Entomol. 99, 1381–1387.

Tinsley NA, Estes RE, Gray ME, 2013. Validation of a nested error component model to estimate damage caused by corn rootworm larvae. J. Appl. Entomol. 137, 161–169.

USDA FAS [United States Department of Agriculture Foreign Agricultural Service], 2012. World Agricultural Production (12-12). United States Department of Agriculture, Washington DC.

USEPA OPPTS [United States Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances], 2011. Pesticide Fact Sheet (SmartStax). United States Environmental Protection Agency, Washington, DC.

Table 3.1 Statistical test[1] of the fixed effect of population for proportional survival on the isoline treatment[2]

Effect	df_N	df_D	F value	P value
Population	1	2.0	28.1	0.03

[1]Statistical test was performed using PROC MIXED of SAS 9.2. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D). Data were analyzed using a square-root transformation. [2]The "isoline" treatment included hybrids without western corn rootworm-active insecticidal toxins.

Table 3.2 Mean[1] proportional survival on the isoline treatment[2] for the effect of population

Population	Proportional survival (%)[3]
Rotation-resistant	52.7 a
Rotation-susceptible	38.7 b

[1]Mean comparisons were performed using PROC MIXED of SAS 9.2. Means within a column that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analyzed using a square-root transformation—actual means are reported. [2]The "isoline" treatment included hybrids without western corn rootworm-active insecticidal toxins. [3]Proportional survival (%) represents the number of larvae recovered divided by the infestation rate (225 eggs/plant).

Table 3.3 Statistical tests[1] of fixed effects for relative corrected mortality

Effect	df_N	df_D	F value	P value
Population	1	17.4	1.9	0.19
Treatment	1	58.7	0.7	0.39
Population × treatment	1	18.6	0.6	0.46

[1]Statistical tests were performed using PROC MIXED of SAS 9.2. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D). Data were analyzed using a square-root transformation.

Table 3.4 Mean[1] relative corrected mortality for the interaction of population and treatment

Population	Treatment[2]	Relative corrected mortality (%)[3]
Rotation-resistant	Single toxin	67.0 a
	Pyramid	71.6 a
Rotation-susceptible	Single toxin	71.1 a
	Pyramid	72.0 a

[1]Mean comparisons were performed using PROC MIXED of SAS 9.2. Means within a column that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analyzed using a square-root transformation—actual means are reported. [2]The "single toxin" treatment included hybrids expressing either Cry34/35Ab1 or Cry3Bb1. The "pyramid" treatment was a hybrid expressing both Cry34/35Ab1 and Cry3Bb1. [3]Relative corrected mortality (%) represents the mortality caused by the single toxin and pyramid treatments, corrected for natural mortality relative to each population.

Table 3.5 Statistical tests[1] of fixed effects for head capsule widths

Effect	df_N	df_D	F value	P value
Population	1	5.2	1.1	0.35
Treatment	2	27.0	64.8	< 0.01
Population × treatment	2	8.6	0.4	0.68

[1]Statistical tests were performed using PROC MIXED of SAS 9.2. The Kenward-Roger adjustment was used to approximate denominator degrees of freedom (df_D). Data were analyzed using a square-root transformation.

Table 3.6 Mean[1] head capsule widths for the interaction of population and treatment

Population	Treatment[2]	Head capsule width (µm)
Rotation-resistant	Isoline	268 a
	Single toxin	235 b
	Pyramid	220 b
Rotation-susceptible	Isoline	261 a
	Single toxin	228 b
	Pyramid	224 b

[1]Mean comparisons were performed using PROC MIXED of SAS 9.2. Means within a column that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analyzed using a square-root transformation—actual means are reported. [2]The "isoline" treatment included hybrids without western corn rootworm-active insecticidal toxins. The "single toxin" treatment included hybrids expressing either Cry34/35Ab1 or Cry3Bb1. The "pyramid" treatment was a hybrid expressing both Cry34/35Ab1 and Cry3Bb1.

CHAPTER 4: VALIDATION OF A NESTED ERROR COMPONENT MODEL TO ESTIMATE DAMAGE CAUSED BY CORN ROOTWORM LARVAE

Introduction

The corn rootworm complex includes three species—the western corn rootworm (*Diabrotica virgifera virgifera* LeConte), the northern corn rootworm (*Diabrotica barberi* Smith & Lawrence), and the southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber). The combined ranges of these diabroticite pests of corn, *Zea mays* (L.), include nearly all of the corn-producing regions of the United States (Gray et al. 2009). The western and northern corn rootworms are the primary species that threaten corn production in the Midwest (Spencer et al. 2009). Metcalf (1986) estimated that yield losses and management costs associated with this pest complex exceed $1 billion annually in the United States. However, Dun et al. (2010) suggested that a more current estimate may be significantly larger. Although all corn rootworm species are native to the western hemisphere, the range of the western corn rootworm has expanded to include portions of Europe (Berger 2001).

Corn rootworms overwinter as eggs that are laid during mid- to late summer (Ball 1957). Larvae begin hatching from late May to early June and immediately begin feeding on corn roots. The larval feeding period typically lasts until late July, although the onset and duration of this period is influenced strongly by seasonal fluctuations in temperature and soil conditions (Bryson et al. 1953). In Illinois, emergence of corn rootworm beetles begins in late June or early July (Steffey and Gray 2009). Emergence of corn rootworm beetles is delayed for Bt corn when compared with non-Bt corn (Crowder et al. 2005; Clark et al. 2012). Males typically emerge one week prior to females (Short and Hill 1972). Mating between beetles occurs during July and August, and teneral females are often mated immediately after emergence (Ball 1957). Although the potential for adults to injure a corn crop exists due to silk feeding (Ball 1957; Culy et al. 1992), injury caused by larvae is widely considered to be the primary manner in which these insects cause yield loss.

Once corn rootworm larvae enter corn roots, they tunnel toward the root tip (Strnad and Bergman 1987). Plant height, root weight, root injury, and yield can be significantly correlated with larval feeding (Owens et al. 1974). Photosynthetic rates are often reduced when larvae begin feeding on roots (Godfrey et al. 1993; Riedell and

Reese 1999; Urías-López et al. 2000). However, the degree of reduction and subsequent compensation may depend on plant phenology (Godfrey et al. 1993) or differences between hybrids (Urías-López et al. 2000), respectively. Root injury may also interfere with the ability of corn plants to utilize soil moisture (Godfrey et al. 1993) and nitrogen (Spike and Tollefson 1989a). Additionally, root injury can lead to colonization by phytopathogenic or saprophytic fungi (Bryson et al. 1953; Kurtz et al. 2010). Environmental factors play a role in determining the severity of root injury. Excessive rainfall and saturated soil conditions prevent root injury (Spike and Tollefson 1988)—soil compaction results in less root injury as well (Ellsbury et al. 1994).

Predicting yield loss caused by corn rootworm larvae has been a goal of many researchers (Branson et al. 1980; Sutter et al. 1990; Tollefson 1990; Levine and Gray 1994). In addition to the physiological symptoms associated with root injury, root lodging can cause harvestability problems—lodged plants are often difficult to harvest using conventional machinery. Severe lodging can reduce grain yield by 11 to 34% (Spike and Tollefson 1991). Yield loss due to root injury or lodging is highly variable and influenced by a number of biotic and abiotic factors (Gray and Steffey 1998). Severity of yield loss due to lodging is related to soil moisture—dry soil conditions increase yield loss and may result in plant death (Bryson et al. 1953; Spike and Tollefson 1989a). Despite root injury, yield may not be affected under conditions of moderate environmental stress or minimal lodging (Cox et al. 2008). When environmental conditions are optimal for corn development, yield loss due to root injury is highly variable (Spike and Tollefson 1989b).

A number of methods have been evaluated for estimating damage from root injury caused by corn rootworm larvae. Root-injury ratings are more sensitive for predicting damage than vertical pulling weight, lodging, stunting, or beetle emergence (Branson et al. 1980). Additionally, Gray and Steffey (1998) noted that root-injury ratings were more closely correlated with yield loss than root volume measurements. Oleson et al. (2005) developed a node-injury scale to quantify root injury. The scale is linear (0 to 3) and based on the proportion of nodal roots that exhibit injury. One complete node of roots injured can result in yield reductions of 0.04, 0.48, and 1.40 t/ha under low, medium, and high stress conditions (Oleson et al. 2005). Dun et al. (2010) suggested that a 17.9% yield loss can be expected for each node of roots injured by corn rootworm larvae. The primary objective of this analysis was to refine and enhance the damage function developed by Dun et al. (2010) to describe the relationship between yield loss and root injury caused by corn rootworm larvae. The method used by Dun et al. (2010) utilized a nested

error component model with unbalanced panel data. Panel data are collected from various groups over time. Because data for all groups may not be present for each time period, data of this type are often nested and may be unbalanced.

Materials and Methods

Data Preparation

Data used for this analysis were from small-plot field experiments conducted throughout Illinois from 2005 through 2011. The experiments were conducted at University of Illinois Research and Education Centers (Figure 4.1). Data were collected by personnel with the Insect Management and Insecticide Evaluation Program (Department of Crop Sciences, University of Illinois). Personnel within this program conduct trials to evaluate both commercial and pre-commercial products for reducing root injury caused by corn rootworms. Results from these experiments are published annually in a report titled *on Target: Annual Review of University of Illinois Insect Management Trials*. In total, data used for this analysis represent 19 location-years. The following paragraphs provide a general overview of the experimental design and data collection techniques for the experiments included in this analysis—for exact dates and specific annual information, see the references listed in Table 4.1.

All experiments were planted from mid-April to late May. Weather conditions during each year largely determined planting date. Prior to planting the experiments, each location received conventional tillage. Seeding rates ranged from 81,500 to 88,900 seeds/ha, and row spacing was always 0.76 m. All experiments were conducted using a randomized complete block design with four replications. Individual plots were always 3.0 m (four rows) wide but ranged in length from 9.1 to 13.7 m. For each experiment, the individual plots received similar fertilization and weed-control practices. As a result, the only factor being evaluated was the root protection product. The previous crop for each experiment was a trap crop (late-planted corn and pumpkins), a practice commonly used for trials evaluating corn rootworm management products.

The methods used to evaluate root injury and to estimate corn yield were identical for all location-years. To quantify injury caused by corn rootworm larvae, five randomly selected roots were extracted from the first row of each plot during mid- to late July. The root systems were washed and rated for injury using the 0 to 3 node-injury

scale developed by Oleson et al. (2005). Yields were estimated by mechanically harvesting the center two rows of each plot after the corn crop reached physiological maturity (dates ranged from mid-September through late November). Harvested weights were converted to t/ha at 15.5% moisture.

To produce panel data for each location-year, the method outlined by Dun et al. (2010) was used. This method produces a dataset containing all possible pairwise comparisons between individual treatments for proportional difference in yield and associated node-injury scale difference. This technique allows for the comparison of the various control products to not only the untreated check(s), but also to every other individual treatment. As in Dun et al. (2010), treatments were paired so that the difference in node-injury scores was always positive; however, the proportional difference in yield could be either positive or negative. The number of observations produced by this method can be summarized by the expression $\sum_{i=1}^{n}(x_i - 1)$ where x_i = the ith treatment number and n = the total number of treatments (including any untreated checks). For example, if a given location-year contained 20 treatments, the expression becomes $\sum_{i=1}^{20}(x_i - 1) = (1-1) + (2-1) + \cdots + (19-1) + (20-1) = 190$. This method produced 7118 observations for proportional yield loss and associated node-injury scale difference. A summary of the numbers of treatments and observations for each location-year is located in Table 4.1.

Statistical Analysis

Because this analysis contains both fixed and random effects, I used the procedure for mixed models (PROC MIXED) in SAS 9.2 (SAS Institute Inc., Cary, NC) to estimate the relationship between proportional yield loss and node-injury scale difference. The model used for this analysis can be represented in matrix notation by the formula $\mathbf{Y} = \mathbf{X\beta} + \mathbf{Z\gamma} + \boldsymbol{\varepsilon}$ where $\mathbf{Y}$ = the known vector of observations for proportional yield loss, $\mathbf{X}$ = the known design matrix for fixed-effect parameters, $\boldsymbol{\beta}$ = the unknown vector of fixed-effect parameters, $\mathbf{Z}$ = the known design matrix for random-effect parameters, $\boldsymbol{\gamma}$ = the unknown vector of random-effect parameters, and $\boldsymbol{\varepsilon}$ = the unknown vector of observational errors. By using PROC MIXED, estimates were obtained for the fixed-effect slope of the relationship between proportional yield loss and node-injury scale difference (β_1) and the random effects of year (σ_Y^2), location nested within year ($\sigma_{L(Y)}^2$), and experimental error (σ_E^2). Littell et al. (1996) provide a comprehensive review of mixed model theory and the calculations used for parameter estimation by PROC MIXED.

PROC MIXED provides a number of techniques that can be used to estimate variance components (i.e., random effects). Method-of-moment techniques (e.g., TYPE1, TYPE2, and TYPE3) use ordinary least squares to estimate variance components (SAS Institute 2009). However, variance component estimates resulting from these techniques may be biased and can result in negative values—variance components are non-negative values by definition (Kuehl 2000). An alternative to method-of-moment techniques are likelihood-based techniques, including maximum likelihood (ML), restricted maximum likelihood (REML), and minimum variance quadratic unbiased estimation (MIVQUE) (SAS Institute 2009). Computationally, MIVQUE is the simplest likelihood-based technique and was used for this analysis.

The covariance test option (COVTEST) was used to produce a parameter estimate, standard error, and Wald Z-test statistic for each variance component (α = 0.05). The solution option (SOLUTION) was used to provide a parameter estimate, standard error, and t-test statistic for the fixed effect slope of the relationship between proportional yield loss and node-injury scale difference (α = 0.05). The suppress intercept option (NOINT) was used to exclude the fixed effect intercept parameter estimate (β_0) from the model. This option forces the relationship between proportional yield loss and node-injury scale difference to include the origin. Dun et al. (2010) also excluded the intercept from their model and suggested that if the node-injury scale difference was zero, no difference in proportional yield loss should be observed. One important implication of excluding the intercept from the model is that the coefficient of determination (R^2) loses its traditional interpretation (Kutner et al. 2004); therefore, the R^2 for this model was not reported. The Kenward-Roger adjustment was used for calculating denominator degrees of freedom (DDFM = KR) (Kenward and Roger 1997).

Results

Data from the field experiments used for this analysis were highly variable across years. Mean node-injury ratings for the untreated check plots ranged from 0.05 (Perry, 2010) to 2.95 (DeKalb, 2005). Yield data ranged from 2.3 t/ha (Urbana, 2006) to 16.9 t/ha (Monmouth, 2009). The variability observed for root injury and yield indicates that this analysis included data from a wide variety of growing conditions, including both minimal and substantial root injury situations with respect to corn rootworm larvae.

Prior to obtaining final parameter estimates, I first ran the model including the intercept term (β_0). As expected, the intercept estimate was not significant (β_0 = 0.0009, t = 0.12, P = 0.91), and the decision to exclude the intercept from the model was valid. The final parameter estimate for the fixed-effect slope of the relationship between proportional yield loss and node-injury scale difference was significant (β_1 = 0.1519, t = 50.23, P < 0.01). This estimate suggests that each unit difference in the node-injury scale results in a yield loss of 15.19%. The relationship between proportional yield loss and node-injury scale difference is represented graphically in Figure 4.2.

Variance component parameter estimates for the random effects of year, location, and experimental error are reported in Table 4.2. The parameter estimate for year (σ_Y^2) was not significant (P = 0.39); however, the parameter estimates for both location ($\sigma_{L(Y)}^2$) and experimental error (σ_E^2) were highly significant (P < 0.01 for both estimates). Numerically, the parameter estimate for experimental error was 28-times greater than the parameter estimate for location.

Discussion

To my knowledge, this is the largest dataset used to estimate a damage function for corn rootworm larvae (7118 observations over 19 location-years). My results are largely consistent with those presented for other experiments examining the relationship between yield loss and root injury. Previous studies suggest yield losses in the range of 6 to 30% for one to two nodes of roots injured (Kahler et al. 1985; Sutter et al. 1990; Spike and Tollefson 1991; Godfrey et al. 1993; Davis 1994; Roth et al. 1995; Riedell et al. 1996; Urías-López and Meinke 2001; Oleson et al. 2005). Unlike this analysis, most of these experiments did not include Bt corn hybrids. Because 67% of all corn planted in the United States contains at least one Bt toxin (USDA ERS 2012), I suggest that this analysis is an important step toward a contemporary understanding of how root injury and yield loss are related. The slope of the relationship between proportional yield loss and node-injury scale difference for this analysis suggests that each unit increase in the 0 to 3 node-injury scale results in a yield loss of 15.19%. Of particular interest was how my results compared with those presented by Dun et al. (2010). Their analysis suggested a 17.9% yield loss per node of roots injured. Because results from these two experiments differed by less than 3%, I suggest that a nested error

component model using panel data is an appropriate and robust approach for describing the damage function of corn rootworm larvae.

The observation that the random effect of year was not statistically significant suggests that, when compared with other effects in the model, the effect of year did not account for a significant amount of variability in proportional yield loss. Dun et al. (2010) also observed a non-significant effect of year in their model and hypothesized that examining data spanning many years (as was done in this analysis) would increase the likelihood of detecting significance. One potential explanation for my inability to observe a significant effect of year may relate to the inclusion of location in the model. Weather can vary significantly not only across years, but also across locations within a single year. I hypothesize that the un-modeled variability due to weather was partitioned into the effect of location rather than the effect of year.

Variability due to the random effect of location nested within year was highly significant. This finding indicates that the placement of a trial geographically was responsible for explaining a significant amount of variability in proportional yield loss. The significant effect of location was not surprising considering that the mean distance between experimental sites was 210 km (Figure 4.1). As previously mentioned, I hypothesize that one of the principal factors contributing to the significant effect of location was variation in weather across locations. The experimental sites were likely separated by enough distance that the various locations may have been subjected to different patterns of temperature or precipitation, both of which may interact to significantly affect corn yield (Hollinger and Angel 2009) and corn rootworm phenology (Wilde 1971; Chiang 1973; Davis et al. 1996).

Another factor that may have contributed to the significant random effect of location is soil type. Soil type can affect both corn yield (Van Doren et al. 1976; Oberle and Keeney 1990) and corn rootworm larval survival (Turpin and Peters 1971; MacDonald and Ellis 1990). The soil profiles for the locations used in this analysis were: (1) DeKalb—El Paso silty clay loam (39%), Flanagan silt loam (33%), Catlin silt loam (17%), and Harper silty clay loam (11%); (2) Monmouth—Muscatine silt loam (74%), Osco silt loam (18%), and Sable silty clay loam (8%); (3) Perry—Downsouth silt loam (69%) and Ipava silt loam (31%); and (4) Urbana—Drummer silty clay loam (56%), Thorp silt loam (33%), and Flanagan silt loam (11%). Although the base soil type for each location was loam, the concentrations of clay and silt present were variable across experimental sites—this may have contributed to the variability associated with the effect of location.

As was the case for the model developed by Dun et al. (2010), the largest source of variability in this model was the experimental error. This implies that after accounting for the fixed effect of root injury and the random effects of year and location nested within year, a significant amount of variability remained unexplained. This observation is not surprising considering the amount of variability in yield and proportional yield loss observed for the experiments. Yields from the raw data ranged from 2.3 to 16.9 t/ha; proportional differences in yield ranged from –122 to 77%. After completing this analysis, I examined proportional yield loss for all observations with a node-injury scale difference of 1.0 (N = 18). According to this model, I would predict a 15% yield loss for such a difference. However, the range in proportional yield loss for these observations was –16 to 69%. This variability highlights the limitations of the damage function I present. It is unlikely that this model could be used successfully to predict yield loss for an individual cornfield or small cluster of cornfields. Rather, this model is better suited to describe the damage function of corn rootworm larvae on a state- or regional-scale over many years.

There are a number of potential explanations for the substantial experimental error. First, since the hybrids evaluated during these experiments were not usually consistent across years, hybrid effects may have contributed to variability in proportional yield loss. Hybrids were provided by a number of different seed companies and likely had very different genetic backgrounds. As a result, I hypothesize that variability in the individual hybrids' yield potential and response to environmental conditions contributed to the highly significant experimental error. At least two experiments have documented variability in hybrid response to root injury caused by corn rootworm larvae (Gray and Steffey 1998; Urías-López and Meinke 2001). Other factors that may have contributed to the substantial experimental error for proportional yield loss include variations across location-years that were not accounted for in the model. These may have included planting date, planting population, plant emergence, crop moisture at harvest, and differences related to the types of management tactics that were evaluated during the experiments.

In my opinion, there are a number of ways in which future analyses of the damage function of corn rootworm larvae could be improved. I suspect that including data from multiple states or regions would enhance this damage function. Corn production can vary greatly throughout the United States and may include practices that do not occur commonly in Illinois (e.g., irrigation, diverse crop rotation, etc.). Dun et al. (2010) observed that the damage functions for corn rootworm larvae in Illinois and Italy differed substantially—one of the primary differences

between these two locations is the use of irrigation in the Italian experiments. Currently, it is unknown whether the damage function for corn rootworm larvae differs for individual corn rootworm species. The predominant species in Illinois is the western corn rootworm. By including data from states or regions where the northern corn rootworm contributes substantially to root injury and yield loss, this damage function may be enhanced. Additionally, including factors in the model that are known to affect both corn yield and corn rootworm larvae may refine this damage function. One example includes seasonal fluctuations in temperature and moisture that deviate from known optimums. Finally, an analysis similar to this one that also incorporated information on the relative performance of the management tactics being evaluated may help constitute a more current, refined estimate of the annual damage associated with these very serious pests of corn.

References

Ball HJ, 1957. On the biology and egg-laying habits of the western corn rootworm. J. Econ. Entomol. 50, 126–128.

Berger HK, 2001. The western corn rootworm (*Diabrotica virgifera virgifera*): a new maize pest threatening Europe. Bull. OEPP 31, 411–414.

Branson TF, Sutter GR, Fisher JR, 1980. Plant response to stress induced by artificial infestations of western corn rootworm. Environ. Entomol. 9, 253–257.

Bryson HR, Wilbur DA, Burkhardt CC, 1953. The western corn rootworm, *Diabrotica virgifera* Lec. in Kansas. J. Econ. Entomol. 46, 995–999.

Chiang HC, 1973. Bionomics of the northern and western corn rootworms. Annu. Rev. Entomol. 18, 47–72.

Clark TL, Frank DL, French BW, Meinke LJ, Moellenbeck D, Vaughn TT, Hibbard BE, 2012. Mortality impact of MON863 transgenic maize roots on western corn rootworm larvae in the field. J. Appl. Entomol. 136, 721–729.

Cox WJ, Shields E, Cherney DJR, 2008. Western corn rootworm damage subtly affects corn growth under moderate environmental stress. Crop Sci. 48, 1164–1169.

Crowder DW, Onstad DW, Gray ME, Pierce CMF, Hager AG, Ratcliffe ST, Steffey KL, 2005. Analysis of the dynamics of adaptation to transgenic corn and crop rotation by western corn rootworm (Coleoptera: Chrysomelidae) using a daily time-step model. J. Econ. Entomol. 98, 534–551.

Culy MD, Edwards CR, Cornelius JR, 1992. Effect of silk feeding by western corn rootworm (Coleoptera: Chrysomelidae) on yield and quality of inbred corn in seed corn production fields. J. Econ. Entomol. 85, 2440–2446.

Davis PM, 1994. Comparison of economic injury levels for western corn rootworm (Coleoptera: Chrysomelidae) infesting silage and grain corn. J. Econ. Entomol. 87, 1086–1090.

Davis PM, Brenes N, Allee LL, 1996. Temperature dependent models to predict regional differences in corn rootworm (Coleoptera: Chrysomelidae) phenology. Environ. Entomol. 25, 767–775.

Dun Z, Mitchell PD, Agosti M, 2010. Estimating *Diabrotica virgifera virgifera* damage functions with field trial data: applying an unbalanced nested error component model. J. Appl. Entomol. 134, 409–419.

Ellsbury MM, Schumacher TE, Gustin RD, Woodson WD, 1994. Soil compaction effect on corn rootworm populations in maize artificially infested with eggs of western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 23, 943–948.

Estes RE, Schroeder JB, Steffey KL, Gray ME, 2005. Evaluation of products to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2005. In: On Target: 2005 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 3–8.

Estes RE, Schroeder JB, Steffey KL, Gray ME, 2006. Evaluation of liquid Force to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2006. In: On Target: 2006 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 19–20.

Estes RE, Steffey KL, Gray ME, 2007a. Evaluation of Force 2.25CS to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2007. In: On Target: 2007 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 16–17.

Estes RE, Steffey KL, Gray ME, 2007b. Evaluation of products to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2007. In: On Target: 2007 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 4–13.

Estes RE, Heeren JR, Tinsley NA, Steffey KL, Gray ME, 2008. Evaluation of Force 2.25CS to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2008. In: On Target: 2008 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 18–19.

Estes RE, Tinsley NA, Gray ME, 2009a. Evaluation of Force 2.1CS and transgenic rootworm hybrids to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2009. In: On Target: 2009 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 14–21.

Estes RE, Tinsley NA, Gray ME, 2009b. Evaluation of soil-applied insecticides plus transgenic rootworm hybrids to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2009. In: On Target: 2009 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 11–13.

Estes RE, Tinsley NA, Gray ME, 2010. Evaluation of soil-applied insecticides plus transgenic rootworm hybrids to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2010. In: On Target: 2010 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 12–14.

Godfrey LD, Meinke LJ, Wright RJ, 1993. Effects of larval injury by western corn rootworm (Coleoptera: Chrysomelidae) on gas exchange parameters of field corn. J. Econ. Entomol. 86, 1546–1556.

Gray ME, Sappington TW, Miller NJ, Moeser J, Bohn MO, 2009. Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54, 303–321.

Gray ME, Steffey KL, 1998. Corn rootworm (Coleoptera: Chrysomelidae) larval injury and root compensation of 12 maize hybrids: an assessment of the economic injury index. J. Econ. Entomol. 91, 723–740.

Hollinger SE, Angel JR, 2009. Weather and crops. In: Illinois Agronomy Handbook. University of Illinois Extension, Urbana, IL, pp. 1–12.

Kahler AL, Olness AE, Sutter GR, Dybing CD, Devine OJ, 1985. Root damage by western corn rootworm and nutrient content in maize. Agron. J. 77, 769–774.

Kenward MG, Roger JH, 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53, 983–997.

Kuehl RO, 2000. Design of Experiments: Statistical Principles of Research Design and Analysis, Second Edition. Brooks/Cole, Belmont, CA.

Kurtz B, Karlovsky P, Vidal S, 2010. Interaction between western corn rootworm (Coleoptera: Chrysomelidae) larvae and root-infecting *Fusarium verticillioides*. Environ. Entomol. 39, 1532–1538.

Kutner MH, Nachtsheim CJ, Neter J, 2004. Applied Linear Regression Models, Fourth Edition. McGraw-Hill, Boston, MA.

Levine E, Gray ME, 1994. Use of cucurbitacin vial traps to predict corn rootworm (Coleoptera: Chrysomelidae) larval injury in a subsequent crop of corn. J. Entomol. Sci. 29, 590–600.

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, 1996. SAS System for Mixed Models. SAS Institute Inc., Cary, NC.

MacDonald PJ, Ellis CR, 1990. Survival time of unfed, first-instar western corn rootworm (Coleoptera: Chrysomelidae) and the effects of soil type, moisture, and compaction on their mobility in soil. Environ. Entomol. 19, 666–671.

Metcalf RL, 1986. Foreword. In: Methods for the Study of Pest *Diabrotica*, ed. Krysan JL, Miller TA. Springer, New York, NY, pp. vii–xv.

Oberle SL, Keeney DR, 1990. Soil type, precipitation, and fertilizer N effects on corn yields. J. Prod. Agric. 3, 522–527.

Oleson JD, Park Y, Nowatzki TM, Tollefson JJ, 2005. Node-injury scale to evaluate root injury by corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 98, 1–8.

Owens JC, Witkowski JF, Tollefson JJ, Rogers RR, Peters DC, 1974. Greenhouse evaluation of soil insecticides for western corn rootworm control. J. Econ. Entomol. 67, 772–774.

Riedell WE, Reese RN, 1999. Maize morphology and shoot CO_2 assimilation after root damage by western corn rootworm larvae. Crop Sci. 39, 1332–1340.

Riedell WE, Schumacher TE, Evenson PD, 1996. Nitrogen fertilizer management to improve crop tolerance to corn rootworm larval feeding damage. Agron. J. 88, 27–32.

Roth GW, Calvin DD, Lueloff S, 1995. Tillage, nitrogen timing, and planting date effects on western corn rootworm injury to corn. Agron. J. 87, 189–193.

SAS Institute, 2009. SAS/STAT 9.2 User's Guide, Second Edition. SAS Institute Inc., Cary, NC.

Short DE, Hill RE, 1972. Adult emergence, ovarian development, and oviposition sequence of the western corn rootworm in Nebraska. J. Econ. Entomol. 65, 685–689.

Spencer JL, Hibbard BE, Moeser J, Onstad DW, 2009. Behaviour and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Agric. For. Entomol. 11, 9–27.

Spike BP, Tollefson JJ, 1988. Western corn rootworm (Coleoptera: Chrysomelidae) larval survival and damage potential to corn subjected to nitrogen and plant density treatments. J. Econ. Entomol. 81, 1450–1455.

Spike BP, Tollefson JJ, 1989a. Relationship of plant phenology to corn yield loss resulting from western corn rootworm (Coleoptera: Chrysomelidae) larval injury, nitrogen deficiency, and high plant density. J. Econ. Entomol. 82, 226–231.

Spike BP, Tollefson JJ, 1989b. Relationship of root ratings, root size, and root regrowth to yield of corn injured by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 82, 1760–1763.

Spike BP, Tollefson JJ, 1991. Yield response of corn subjected to western corn rootworm (Coleoptera: Chrysomelidae) infestation and lodging. J. Econ. Entomol. 84, 1585–1590.

Steffey KL, Gray ME, 2009. Managing insect pests. In: Illinois Agronomy Handbook. University of Illinois Extension, Urbana, IL, pp. 179–196.

Strnad SP, Bergman MK, 1987. Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. Environ. Entomol. 16, 1193–1198.

Sutter GR, Fisher JR, Elliott NC, Branson TF, 1990. Effect of insecticide treatments on root lodging and yields of maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 83, 2414–2420.

Tinsley NA, Estes RE, Gray ME, 2009. Evaluation of products to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2009. In: On Target: 2009 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 4–10.

Tinsley NA, Estes RE, Gray ME, 2010. Evaluation of products to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2010. In: On Target: 2010 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 5–11.

Tinsley NA, Estes RE, Gray ME, 2011. Evaluation of products to control corn rootworm larvae (*Diabrotica* spp.) in Illinois, 2011. In: On Target: 2011 Annual Summary of Field Crop Insect Management Trials. University of Illinois Extension, Urbana, IL, pp. 4–10.

Tollefson JJ, 1990. Comparison of adult and egg sampling for predicting subsequent populations of western and northern corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 83, 574–579.

Turpin FT, Peters DC, 1971. Survival of southern and western corn rootworm larvae in relation to soil texture. J. Econ. Entomol. 64, 1448–1451.

Urías-López MA, Meinke LJ, 2001. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on yield of different types of maize. J. Econ. Entomol. 94, 106–111.

Urías-López MA, Meinke LJ, Higley LG, Haile FJ, 2000. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on photosynthetic rate and vegetative growth of different types of maize. Environ. Entomol. 29, 861–867.

USDA ERS [United States Department of Agriculture Economic Research Service], 2012. Adoption of Genetically Engineered Crops in the US. United States Department of Agriculture, Washington DC.

Van Doren DM, Triplett GB, Henry JE, 1976. Influence of long term tillage, crop rotation, and soil type combinations on corn yield. Soil Sci. Soc. Am. J. 40, 105–108.

Wilde GE, 1971. Temperature effect on development of western corn rootworm eggs. J. Kansas Entomol. Soc. 44, 185–187.

Figure 4.1 Location of University of Illinois Research and Education Centers (1 cm ≈ 48 km)

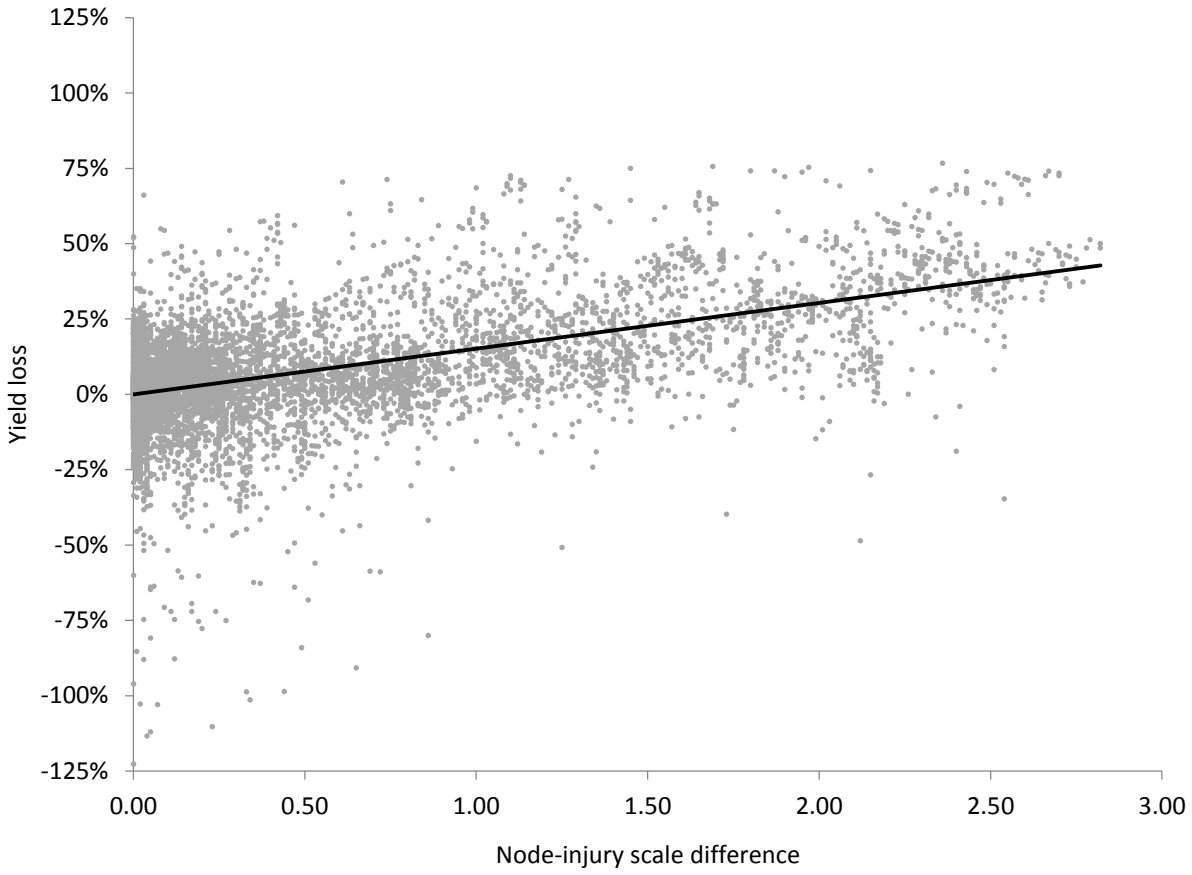

Figure 4.2 Regression relationship between proportional yield loss and node-injury scale difference (β_1 = 0.1519, PROC MIXED, SAS 9.2)

Table 4.1 Number of treatments and observations for each location-year with references (2005–2011)

Year	Location	Treatments	Observations	Reference(s)
2005	DeKalb	27	351	Estes et al. 2005
	Monmouth	26	325	Estes et al. 2005
2006	Urbana	14	91	Estes et al. 2006
2007	DeKalb	27	351	Estes et al. 2007b
	Monmouth	23	253	Estes et al. 2007b
	Perry	20	190	Estes et al. 2007b
	Urbana	37	666	Estes et al. 2007a, 2007b
2008	Urbana	9	36	Estes et al. 2008
2009	DeKalb	43	903	Estes et al. 2009a, 2009b; Tinsley et al. 2009
	Monmouth	42	861	Estes et al. 2009a, 2009b; Tinsley et al. 2009
	Perry	35	595	Estes et al. 2009a; Tinsley et al. 2009
	Urbana	43	903	Estes et al. 2009a, 2009b; Tinsley et al. 2009
2010	DeKalb	26	325	Estes et al. 2010; Tinsley et al. 2010
	Perry	22	231	Tinsley et al. 2010
	Urbana	27	351	Estes et al. 2010; Tinsley et al. 2010
2011	DeKalb	20	190	Tinsley et al. 2011
	Monmouth	18	153	Tinsley et al. 2011
	Perry	18	153	Tinsley et al. 2011
	Urbana	20	190	Tinsley et al. 2011

Table 4.2 Statistical tests[1] of variance component parameter estimates for random effects

Effect	Parameter	Estimate	Standard error	Wald Z value	P value
Year	σ_Y^2	4.3×10^{-5}	1.5×10^{-4}	0.3	0.39
Location	$\sigma_{L(Y)}^2$	8.5×10^{-4}	1.9×10^{-4}	4.6	< 0.01
Experimental error	σ_E^2	2.4×10^{-2}	4.0×10^{-4}	59.5	< 0.01

[1]Statistical tests were performed using PROC MIXED of SAS 9.2.